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0 0301 0021036 5

CHEMICAL EMBRYOLOGY

IN THREE VOLUMES

VOL. I

New York
The Macmillan Co.
London
The
Cambridge University Press
Bombay, Calcutta and
Madras
Macmillan and Co., Ltd.
Toronto
The Macmillan Co. of
Canada, Ltd.

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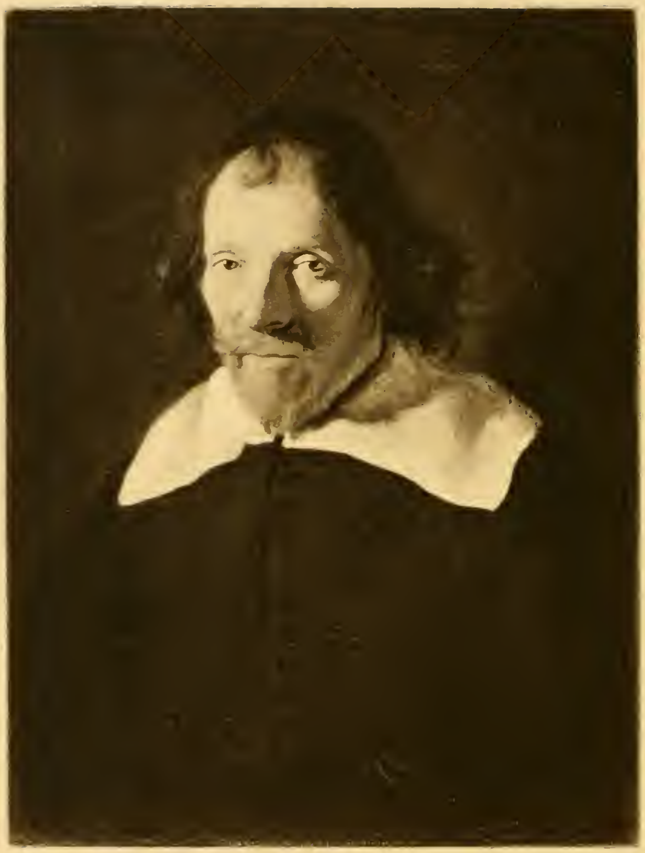

Vir Praeclarissimus
GULIELMUS HARVEY.
Mysterii Generationis Indagator Diligens.

CHEMICAL EMBRYOLOGY

BY

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VOLUME ONE

NEW YORK: THE MACMILLAN COMPANY
CAMBRIDGE, ENGLAND: AT THE UNIVERSITY PRESS
1931

PRINTED IN GREAT BRITAIN

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in Univ. Aberdon. Anat. olim Professori

FREDERICO GULIELMO SANDERSON
Schol. Undel. olim Praeposito

HUGONI KERR ANDERSON
Coll. Gonv. et Caii olim Custodi

FREDERICO GOWLAND HOPKINS
Artis chemicae ad animantia spectantis in
Univ. Cantabrig. Professori

et

DOROTHEAE MOYLE

hanc suam disquisitionem
auctor
sacram voluit

For more, and abler operations are required to the Fabrick and erection of Living creatures, than to their dissolution, and plucking of them down: For those things that easily and nimbly perish, are slow and difficult in their rise and complement.

William Harvey, *Anatomical Exercitations concerning the generation of living creatures*, London, 1653, Ex. XLI, p. 206.

That discouraging Maxime, Nil dictum quod non dictum prius, *hath little room in my estimation, nor can I tye up my belief to the Letter of Solomon; I do not think, that all Science is Tautology; these last Ages have shown us, what Antiquity never saw; no, not in a dream.*

Joseph Glanville, *Scepsis Scientifica, an essay of the vanity of dogmatizing, and confident opinion*, London, 1661, Chap. XXII.

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ACKNOWLEDGEMENTS OF INDEBTEDNESS

THOSE who have assisted me in the preparation of this work are so numerous that it is impossible to mention them all by name. Its original impetus was derived from a discussion with Professor Sir F. G. Hopkins in 1923 on the observation of Klein that inositol, though absent from the undeveloped hen's egg, was present in considerable quantity at hatching; and throughout the period of preparation his encouragement, help, and advice were never-failing. I have derived great benefit from the discussion of various points with Miss Marjory Stephenson, M. Louis Rapkine, Dr R. A. Fisher, and my wife. Professor J. T. Wilson has been repeatedly helpful to me on anatomical points, and in the Zoological Laboratory I was always sure of obtaining expert advice from Mr James Gray, Mr J. T. Saunders, Mr C. F. A. Pantin and Dr Eastham. I have relied much upon the kindness and wide biological knowledge of Dr D. Keilin and Dr F. H. A. Marshall. As regards the historical chapters, I am most grateful to Dr Charles Singer, who annotated them with valuable comments, and to Professor R. C. Punnett who placed unreservedly at my disposal his knowledge of the history of generation, and his library of old and rare biological books. To Dr Arthur Peck I am indebted for the correction of my Greek, and it was Professor A. B. Cook who guided me to the embryology of the ancients. Without the assiduous backing of Mr Powell, the Librarian of the Royal Society of Medicine, and his assistants, I should have dealt much more inadequately than I have with the papers which cannot be consulted in Cambridge. I have also to thank the administrators of the Thruston Fund of Gonville and Caius College for a grant which was devoted to incidental expenses. For the indexes I wish to thank Miss Helen Moyle, and for other services which have made the book possible, Mrs V. Townsend. My thanks are also due to the Editors of the following journals: *Biochemical Journal*, *Journal*

of Experimental Biology, *Biological Reviews*, *Science Progress*, and the *Monist*, for permission to reprint passages from papers. I must record my gratitude to the following friends, who very kindly read through and criticised the proofs of the various sections:

Part I

Professor A. E. Boycott
Dr J. H. Woodger

Part II

Professor R. C. Punnett
Dr Charles Singer
Dr Reuben Levy
Dr Arthur Peck
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Epilegomena

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Mr G. R. de Beer
Professor A. R. Moore & Mrs Moore

Appendix III

Dr L. E. S. Eastham

I am indebted to the Master of Gonville and Caius College for permission to reproduce the portrait of William Harvey (attributed to Rembrandt) in the Senior Combination Room. Finally, I am glad to record here my gratitude to the Staff of the Cambridge University Press for the unremitting care which they gave to my book during the course of its preparation.

J. N.

NOTE: The use of the shortened and (&) indicates collaboration between two or more authors.

PROLEGOMENA

The Sciences, unlike the Graces or the Eumenides, are not limited in number. Once born, they are immortal, but, as knowledge increases, they are ever multiplying, and so great is now the dominion of the scientific mind that every few years sees a new one brought into the world. Some spring, fully armed, from the brains of one or two men of genius, but most of them, perhaps, come only gradually to their full development through the labours of very many obscure and accurate observers.

If the analogy may be permitted, physico-chemical embryology has so far been living an intra-uterine existence. Its facts have been buried in a wide range of scientific journals, and its theories have lain dormant or *in potentia* in reviews of modest scope. Physico-chemical embryology has, indeed, arrived at the stage immediately prior to birth, and all it needs is a skilful obstetrician, for, when once it has reached the light of day and has passed for ever out of the foetal stage, it will be well able to take care of itself. This obstetrical task is that which I have chosen and obviously enough it divides into three principal heads: first, to collect together out of all the original papers on the subject the facts which are known about the physico-chemical basis of embryonic development; second, to relate these facts to each other and to the facts derived from the labours of investigators in morphological embryology and "Entwicklungsmechanik," and, third, to ascertain whether, from what is at present known, any generally valid principles emerge.

I may as well say at the outset that in order to do this certain arbitrary boundary-lines are inevitable. The following arrangement has been adopted. Chronologically speaking, the prelude to all embryonic development is the maturation of the egg-cell, but this is not strictly embryology, and so has been relegated to an appendix. The egg-cell as a physico-chemical system is dealt with at the opening of Part III, and thereafter the physico-chemical aspects of development follow in order. No mention will be made of fertilisation, for this has been treated exhaustively by other writers (Lillie, Dalcq) and, after all, embryology presupposes fertilisation whether natural or artificial. Nor in later chapters will any complete treatment be given of the events going on in the maternal organism during pregnancy: for the present purpose the discussion will go as far into the mother as the placenta but no farther. Again, hatching or birth will put an end to the discourse as to the foetal state itself, save that, in the cases of animals which hatch before the yolk-sac is absorbed, their embryonic life is assumed to end when they first take food for themselves. Appendices are added dealing with the plant embryo and the insect pupa, which, in the later stages of metamorphosis, have points both of resemblance to and of difference from the growth of the embryo. It is natural to hope that the outcome of all this labour may be an increase of interest among biologists in this section of their domain, and a great accession to the number of those investigators who devote their energies to actual experiments in this new field.

For it must be confessed that it is a new field. It has been opened up in very gradual stages: fitful and sporadic experiments on the constitution of embryonic tissues in the seventeenth century, a gradual growth of knowledge about the chemical composition of eggs in the eighteenth, a big increase of activity in the early nineteenth, during which appear the first observations on the physico-chemical changes taking place in the embryo during its development, and then in our own time a mass of very widely scattered work bringing the subject up to the "obstetrical" stage. Such a work as this, in my opinion, should not be compared with laboratory experiments in a derogatory sense, for, while it is true that facts are the ultimate court of appeal in any scientific discussion, yet at the same time the number of investigators has grown to such extraordinary proportions in this century that some danger exists lest we should be so busily engaged in accumulating new facts as to be left with no time at all to devote any thought to those we have already. Classification, indexing, and maturer consideration about the facts we actually possess are at least as great a need at the present moment as the invention of new facts. "Everyone must realise", says Eugenio Rignano, "how much this theoretical elaboration, performed by means of analyses and comparisons, of generalisations and hypotheses controlled and verified by the correspondence of facts with the results of the reasoning, is useful and necessary if one wishes to reach a progressive systematisation and an ever more synthetic vision of the confused mass of facts which experimentalists pour daily in a continuous stream into the scientific market."

My predecessors in this work have been few in number. The volumes of Haller's, Buffon's, and Milne-Edwards' great treatises, in which they deal with the phenomena of generation, contain as much information as was available up to 1863, but this is purely of historical interest to us. In 1885, W. Preyer, Professor of Physiology at Jena, published his *Spezielle Physiologie des Embryo*, which still remains a most valuable review, and indeed, even to-day, is the only existing book specially devoted to embryonic physiology. The present century has produced only three books which even touch upon my subject, namely, T. B. Robertson's *Chemical Basis of Growth and Senescence*, F. H. A. Marshall's *Physiology of Reproduction* and E. Fauré-Fremiet's *La Cinétique du Développement*. The first of these was admittedly written to support a particular theory, and in any case says comparatively little about physico-chemical embryology. The second and the third deal with it only as a constituent part of a much wider field. In Marshall's case, the whole array of facts relating to oestrus and breeding, fertilisation and fertility, lactation and sex determination, have to be dealt with, and only three chapters out of sixteen are devoted to the subject of this book. The first of these is contributed by W. Cramer, and covers the biochemistry of the sexual organs, including the unfertilised egg; the second, which deals with foetal nutrition and the placenta, is by J. Lochhead; and the third, by these two investigators together, is concerned with changes in the maternal organism during pregnancy. Admirable as these chapters are, they are now rather out of date. Moreover, though one or two corners of the field I have before me were covered in Marshall's book, it was from a quite different standpoint.

Fauré-Fremiet's work is exactly analogous; it deals with physico-chemical embryology only, as it were, in passing. The relevant discussion takes up only two chapters out of seven; the rest are occupied with tissue culture, growth of protozoal populations, and general cytology. His book covers, it might be said, the third and fourth corners: all the main expanse of the field remains.

Thus neither of these books deals with physico-chemical embryology in an exhaustive and comprehensive fashion, treating it as, in my view, it ought to be treated, with the thoroughness which is deserved by a new branch of natural knowledge. Inseparable, however, from thoroughness of treatment is the submergence of the parts of more general interest under a mass of detail, and it may be well, therefore, to mention now what sections of the book could be said to be most valuable to any student of general biology. Part I comes in this class, and of Part III, the middle portion of Section I, all of Sections 2, 3, and 5, the latter half of Section 7, Sections 8, 9 (especially the end), 11, possibly 18, and finally the Epilegomena.

For my models in the preparation of this book, if it is permissible to name them, I have taken, *Growth and Form* by d'Arcy Thompson, surely the most scholarly work produced by a biologist in our time, and *The Physiology of Reproduction* by F. H. A. Marshall, already mentioned, which showed to all successors, in my opinion, how a colossal array of facts can be welded together into an absorbing and readable book. I am conscious that I shall not attain the level of these classics of modern biology, but then

....Pauci, quos aequus amavit
Jupiter, aut ardens evexit ad aethera virtus.

The progress of any branch of natural knowledge can be best described as a continual pilgrimage towards the quantitative. Qualities can never be altogether left out of account and this is what makes it impossible for science to achieve its end with absolute finality. Yet an association with the probably unattainable is common to all the great types of man's activity. But "Fuyez toujours les à peu près", as O. W. Holmes used to put it, is a proper maxim for the scientific mind, and whatever this book can do towards making embryology an exact science will be its final justification.

PART I

THE THEORY OF CHEMICAL EMBRYOLOGY

. . . . to measure all things that can be measured, and to make measurable what cannot yet be measured.

Galileo.

MARINE BIOLOGICAL L
WOODS
LI
MASS.

THE THEORY OF CHEMICAL EMBRYOLOGY

Philosophy, Embryology, and Chemistry

The penetration of physico-chemical concepts into embryology has not been entirely peaceful. "In experimental embryology", it has been said, "concepts borrowed from the physical sciences do not admit of calculations being made, and until they do they are not really playing the same rôle as they do in the sciences from which they have been borrowed and for which they were devised." "Nothing is more clear", says another writer, "in chemistry and physics than that identical results follow upon identical causes. Introduce a disturbing element, even a small one, into your experiment, and the experiment will fail. Such is not the case with the developing egg." W. McDougall, too, endows the egg with good intentions. "The embryo", he says, "seems to be resolved to acquire a certain form and structure, and to be capable of overcoming very great obstacles placed in its path. The development of the forms of organisms seems to be utterly refractory to explanation by mechanical or physico-chemical principles." Finally, J. A. Thomson goes farther than them all, and does not hesitate to say, "It is a mere impious opinion that development will one day be described in terms of mechanics". Chapter IV of his Gifford Lectures illustrates the antagonistic attitude to physico-chemical embryology in its most acute form.

It can hardly be a coincidence that so many among the great embryologists of the past were men of strongly philosophic minds. It would be absurd to support this opinion by citing Aristotle, but it holds less obviously true of William Harvey, whose book on generation is full of thoughts about causation, and in the cases of Ernst von Baer, Ernst Haeckel, Wilhelm Roux, Hans Driesch, d'Arcy Thompson and J. W. Jenkinson, there is no doubt about it. It is not really surprising, for of all the strange things in biology surely the most striking of all is the transmutation inside the developing egg, when in three weeks the white and the yolk give place to the animal with its tissues and organs, its batteries of enzymes and its delicately regulated endocrine system. This coming-to-be can hardly have failed to lead, in the minds of those most intimately acquainted with it, to thoughts of a metaphysical character. Nor, it seemed, did those who worked on it do much to diminish its wonder. "Neither the schools of physicians", as Harvey said, "nor Aristotle's discerning brain, have disclosed the manner how the Cock and its seed, doth mint and coine, the chicken out of the egg." Or, in the words of Erycius Puteanus, "I will neglect gold, and will praise what is more precious than any metal, I will despise feasts, and will set forth praises of something better than any food or drink. If you would know of what it is that I intend to speak, it is the egg; men marvel at the sun, at meteors flung from heaven, at stars swimming therein, but this is the greatest of all wonders". Here, however, there is one significant thing. It is that the very chapter of Harvey's book in which the preceding remark is found has as its heading "The Efficient Cause of the Chicken, is hard to be found out". It certainly was, but the right clue was in the heading to that exercitation.

This close association of embryology with philosophy, then, made it necessary to discuss at the outset of this book certain points in the more theoretical regions of biology, and, as it were, to defend from a theoretical angle the extension of the domain of physics and chemistry over embryology. I might have entitled this part of the book "The philosophy of embryology", but, in deference to those metaphysicians who rightly insist that the word philosophy should only be used of a definite system of experience which looks at the universe as a corporate whole, I adopted the present heading. Under it I propose to discuss the exact status of the chemical aspect of embryology. For many biologists, having perhaps insufficiently considered the nature of the scientific method, think it likely that the discoveries of modern times may allow of some other basis for biology than mathematical physics and that the scientific method may rightly be different in biology from what it is in chemistry. It is this factor in our present intellectual climate which makes it necessary to preface by a philosophical discussion a book in which the concepts of physics and chemistry are extended to a field of biology where they have never before received more than a conventional and formal reverence.

The aim of all studies in physico-chemical embryology must be that expressed by T. H. Huxley when he said, "Zoological Physiology is the doctrine of the functions or actions of animals. It regards animal bodies as machines impelled by certain forces and performing an amount of work which can be measured and expressed in terms of the ordinary forces of nature. The final object of physiology is to deduce the facts of morphology on the one hand and those of oecology on the other hand from the laws of the molecular forces of matter". It may be regarded as very noteworthy that Huxley here puts morphology as secondary to physiology and as it were derivable from it; he does not place morphology and physiology on two high places, "neither afore or after other", as has so often been done, but he plainly states his view that the anatomical aspect of animals, their external and internal forms, could be deduced from the interplay of physico-chemical forces within them, if we only knew enough about those forces. This is the idea of the primacy of function. It seems always to have two meanings, firstly, the Epicurean-Lucretian one which Huxley adopts here and Roux so brilliantly developed, in which shape is regarded as the outward and visible sign of the properties of matter itself, and, secondly, the Aristotelian one emphasised by J. B. de Lamarck's writings in the eighteenth century, and in our time by E. S. Russell's great work *Form and Function*, in which psychical factors are introduced as the essential elements in the ultimate analysis of shape. In both these interpretations, function has the priority over form, but the meaning of function is the point of difference. Some biologists, however, seem to think that physiology and morphology are categorical, and the latter is emphatically not reducible to or derivable from the former. The two spheres of study represent, for them, correlative and immiscible disciplines, morphology aiming ultimately at solid geometry, physiology at causation, and "rerum cognoscere causas" is not the basic desire of the scientific mind. They object to the view which regards "the ovum as a kind of chemical device wound up and ready to go off on receipt of a stimulus, the task of the causal morphologist being to disentangle the complex of events which constitute the unwinding process" (Woodger), complaining that in this view no account is taken of the past history of the race, which is left to genetics, again a causal discipline. To some extent these opinions spring from a conviction that the analytical method is inapplicable to a living being because it is an organism, and of that there is more to be said. But they also arise from a profound unwillingness to subsume biology under physics and a desire to uphold "the autonomy of biology". This precludes the promise of an ever-increasing homogeneity in the structure of science, and hence an ever-increasing simplicity.

The Historical Perspective

That the older embryologists awaited the extension of physico-chemical conceptions to embryology is no mere matter of conjecture. Until the mechanical theory of the universe had been consolidated by the "corpuscularian philosophy" of the seventeenth century it would be useless to look for illustration of this, but by 1674 John Mayow was tracing the part played by the "nitro-aerial particles" in the development of the embryo, and in 1732 Hermann Boerhaave was discussing chemical problems with explicit reference to embryonic development. Many other examples of this point of view in the eighteenth century will be given later. Then, when the second decade of the nineteenth century had nearly gone, von Baer, perhaps the greatest of all embryologists, was careful to preface his *Entwicklungsgeschichte* by a careful account of all that was known about the chemical constitution of the egg, and that, although his philosophical inclinations were deeply vitalistic, and even his practical interests morphological. In Roux, of course, this future reference came out explicitly, and the extension of biochemistry into embryology was allowed for and foreseen. An early instance was the association between Wilhelm His and Hans Miescher. Miescher, writing to Hoppe-Seyler in 1872 said, "I am now collecting material from fishes, birds, and amphibia to lead to a chemical statics of development. With this end in view I shall do analyses of ash, nuclein, and lecithin".

Embryology before Harvey, however, was rigidly Aristotelian, a statement the meaning of which George Santayana has lucidly explained. "Aristotle", said he, "distinguished four principles in the understanding of Nature. The ignorant think that these are all, equally, forces producing change, and the cooperative sources of all natural things. Thus, if a chicken is hatched, they say that the Efficient Cause is the warmth of the brooding hen, yet this heat would not have hatched a chicken out of a stone, so that a second condition, which they call the Material Cause, must be invoked as well, namely, the nature of an egg; the essence of eggness being precisely a capacity to be hatched when warmed gently—because, as they wisely observe, boiling would drive away all potentiality of hatching. Yet, as they further remark, gentle heat-in-general joined with the essence-of-eggness would produce only hatching-as-such and not the hatching of a chicken, so that a third influence, which they call the Final Cause, or the End-in-view, must operate as well, and this guiding influence is the divine idea of a perfect cock or a perfect hen presiding over the incubation and causing the mere eggness in the egg to assume the likeness of the animals from which it came. Nor, finally, do they find that these three influences are sufficient to produce here and now this particular chicken, but are compelled to add a fourth, a Formal Cause, namely, a particular yolk, a particular shell, and a particular farmyard, on which and in which the other three causes may work, and laboriously hatch an individual chicken, probably lame and ridiculous despite so many sponsors." The Aristotelian account of causation could not be better expressed. Santayana puts this description of it into the mouth of Avicenna in his imaginary dialogue, and makes him go on to say, "Thus these learned babblers would put nature together out of words, and would regard the four principles of interpretation as forces mutually supplementary combining to produce material things; as if perfection could be one of the sources of imperfection or as if the form which things happen to have could be one of the causes of their having it. Far differently do these four principles clarify the world when discretion conceives them as four rays shed by the light of an observing spirit". In this last observation we may perhaps trace the germ of the Copernican revolution in philosophy effected by Kant, if we may take it to enclose the idea of the activity of the experient subject in all perception.

In science generally, however, the Aristotelian conceptions went without serious contradiction, and thus formed the framework for all the embryological work that was done, as, for instance, by Albertus Magnus. Owing to its association with the idea of the plan of a divine being, the final cause tended in the Middle Ages to eclipse the others. In the seventeenth century this feeling is well shown in a remarkable passage, which occurs in the *Religio Medici* of Sir Thomas Browne: "There is but one first cause, and four second causes of all things; some are without Efficient, as God; others without Matter, as Angels; some without Form, as the first matter; but every Essence created or uncreated, hath its Final cause, and some positive End both of its Essence and Operation; this is the cause I grope after in the works of Nature; on this hangs the providence of God; to raise so beauteous a structure as the World and the Creatures thereof, was but his Art; but their sundry and divided operations, with their predestinated ends, are from the Treasure of his Wisdom. In the causes, nature, and affections of the Eclipses of the Sun and Moon there is most excellent speculation, but to profound farther, and to contemplate a reason why his providence hath so disposed and ordered their motions in that vast circle as to conjoyn and obscure each other, is a sweeter piece of Reason and a diviner point of Philosophy; therefore sometimes, and in some things, there appears to me as much Divinity in Galen his books *De Usu Partium*, as in Suarez' *Metaphysicks*: Had Aristotle been as curious in the enquiry of this cause as he was of the other, he had not left behind him an imperfect piece of Philosophy but an absolute tract of Divinity". This was written in Harvey's time, and in Harvey's thought the four causes were still supreme; his *De Generatione Animalium* is deeply concerned with the unravelling of the causes which must collaborate in producing the finished embryo. But the end of their domination was at hand, and the exsuccous Lord Chancellor, whose writings Harvey thought so little of, was making an attack on one of Aristotle's causes which was destined to be peculiarly successful. There is no need to quote his immortal passages about the "impertinence", or irrelevance, of final causes in science, for they cannot but be familiar to all scientific men. Bacon demonstrated that from a scientific point of view the final cause was a useless conception; recourse to it as an explanation of any phenomenon might be of value in metaphysics, but was pernicious in science, since it closed the way at once for further experiments. To say that embryonic development took the course it did because the process was drawn on by a pulling force, by the idea of the perfect adult animal, might be an explanation of interest to the metaphysician, but as it could lead to no fresh experiments, it was nothing but a nuisance to the man of science. Later on, it became clear also that the final cause was irrelevant in science owing to its inexpressibility in terms of measurable entities. From these blows the final cause never recovered. In England the seventeenth century was the time of transition in these affairs, and in such books as Joseph Glanville's *Plus Ultra* and *Scepsis Scientifica*, for instance, and Thomas Sprat's *Defence of the Royal Society*, the stormy conflict between the "new or experimental philosophy" and the Aristotelian "school-philosophy" can be easily followed. Francis

Gotch has given a delightful account of the evening of Aristotelianism, but it involved a stormy sunset, and the older ideas did not give way without a struggle. Harvey's work is perfectly representative of the period of transition, for, in his preface under the heading "Of the Method to be observed in the knowledge of Generation", he says, "Every inquisition is to be derived from its Causes, and *chiefly* from the Material and Efficient". As for the formal cause, Bacon expressly excluded it from Physic, and it quietly disappeared as men saw that scientific laws depended on the repeatableness of phenomena, and that anything unique or individual stood outside the scope of science. Thus in the case of the developing egg, the formal (the particular farmyard, etc.) and the final causes are scientifically meaningless, and if it were desired to express modern scientific explanation in Aristotelian terminology, the material and efficient causes would alone be spoken of, essence-of-eggness being a "chymical matter" as well as the heat of the brooding hen.

Obstacles to Chemical Embryology

The complexity of living systems, however, is such that many minds find it difficult to accept this physico-chemical account as the most truly scientific way of looking at it. This is doubtless due in part to an erroneous notion, which is yet very tenacious of existence, that the mechanical theory of the universe must, if accepted at all, be accepted as an ultimate ontological doctrine, and so involve its supporter in one of the classical varieties of metaphysical materialism. It cannot be too strongly asserted that this is not the case. To imagine that it is, is to take no account of the great space that separates us from the last century. "When the first mathematical, logical, and natural uniformities", said William James, "the first Laws, were discovered, men were so carried away by the clearness, beauty, and simplification that resulted that they believed themselves to have deciphered authentically the eternal thoughts of the Almighty. His mind also thundered and reverberated in syllogisms. He also thought in conic sections, squares, and roots and ratios, and geometrised like Euclid. He made Kepler's laws for the planets to follow, he made velocity increase proportionately to the time in falling bodies; he made the laws of the sines for light to obey when refracted; he established the classes, orders, families, and genera of plants and animals, and fixed the distances between them."

Far different is the account of itself which science has since learned to give. But this change of attitude is not a revolt against thought as such, or against reason as such; it is only a loss of belief in the literal inspiration of the formulae proper to science. It would be just as extravagant to claim that the scientific investigator of the twentieth century sets down absolute truths in his laboratory notebook, and, armed with an infallible method, explores the real structure of an objective world, as it would be fantastic to claim that Jehovah dictated an absolute code of the good to Moses on Mount Sinai. To say that the development of a living being can best be described in a metrical or mechanical way is not to say that it *is* metrical or mechanical and nothing else. The physico-chemical embryologist is not committed to any opinion on what his material really is, but he is committed to the opinion that the scientific method is one way of describing it, and that it is best to apply that method in its full rigour if it is to be applied at all. In other words, following the train of thought of William James, he does not assert that the courts of Heaven as well as those of our laboratories resound with expressions such as "organisers of the second grade," and "so many milligrams per cent." The mechanical theory of the world, which is, as many believe, bound up indissolubly with one of the ultimate types of human experience, can no longer be considered as necessarily involving the exclusion of other theories of the world. Or, put in another way, it is a theory of the world, and not a pocket edition of the world itself.

But before bringing forward any arguments in support of this attitude and in defence of physico-chemical embryology, it will be well to consider briefly those theoretical tendencies in modern biology which go together under the inexact adjective "neo-vitalistic", for their influence in scientific thought has been far-reaching. To deal critically with them is not a waste of time, for, were we to adopt any one of them, we should find that the notion of embryology as complicated biophysics and biochemistry would have to be abandoned, and quite other means of approach (never, indeed, very well defined) would have to be used.

The Stumbling-block of Hormism

Hormism, or "Psychobiology," may be dealt with in a few words. Chiefly supported by A. Wagner in Germany, and by E. S. Russell and L. T. Hobhouse in this country, it holds that—to use Lloyd Morgan's terminology—a physiological tale cannot be told separately from a psychological tale. Instead of expressing living processes in terms of physical causes and effects, the hormists wish to regard unconscious striving as the essential urge in life, and such conceptions as food, rest, fatigue, etc., as irreducible biological categories. These thinkers do not often acknowledge their debt to Galen of Pergamos, who put forward, as early as A.D. 170, an essentially similar conception as the basis of his biology. In the treatise *On the Natural Faculties* he says, "The cause of an activity I term a faculty.... Thus we say that there exists in the veins a blood-making faculty, as also a digestive faculty in the stomach, a pulsatile faculty in the heart, and in each of the other parts a special faculty corresponding to the function or activity of that part". He also said, "We call it a faculty so long as we are ignorant of the cause which is operating", but he never actually suggested any such underlying cause, and seems to have thought it impossible to ascertain. So do the hormists. According to them the actions of protozoa are to be described in terms of avoiding responses, seeking responses and the like, language which, as they claim, is much simpler than the complex terminology of surface tension and molecular orientation. Everything, of course, depends on what is meant by simple. To say that a protozoon seeks the light is evidently more naïve than to say that a dimolecular photochemical reaction takes place in its protoplasm leading to an increase of lactic acid or what not on the stimulated side, but since the latter explanation fits into the body of scientific fact known already it is open to the biochemist to say that, for his part, he considers the latter explanation the simpler. It is, in fact, simpler *in the long run.* Psychobiology or hormism differs from the other forms of neo-vitalism because it insists on retaining "commonsense" explanations in biology as categories of biological thought beneath which it is impossible to go. It dismisses the entelechy of dynamic Teleology, on the ground that it acts, as it were, in addition to the mechanistic schema, accepting the latter fully but interfering in it. It resembles much more finalism and organicism, but lays stress rather on the unconscious striving force which seems to animate colloidal solutions of carbohydrates, fats, and proteins. It resembles the Behaviourism of J. B. Watson superficially by emphasising animal behaviour, but it fundamentally differs, for it asks the question—Does an animal see the green light and the red light in this experiment as we do, or does it see them as two shades of grey as colour-blind people do? while the behaviourist asks—Does it respond according to difference of light-intensity or difference of wave-lengths? Hormism, in fact, recurs continually to psychical factors. Samuel Butler, for instance, one of its principal exponents, wrote, "I want to connect the actual manufacture of the things a chicken makes inside an egg with the desire and memory of the chicken so as to show that one and the same set of vibrations at once change the universal substratum into the particular phase of it required" (cf. *ἡ τοῦ δυνάμει ὄντος ἐντελέχεια ᾗ τοιοῦτον*) "and awaken a consciousness of and a memory of and a desire towards this particular phase on the part of the molecules which are being vibrated into it". "The Hormist contends", says Lloyd Morgan, "that something which is very difficult to distinguish from a 'plan-in-mind' on the part of the embryo chick or rabbit does freely determine the course of events in specific growth from egg to adult. This, I urge, is a metaphysical hypothesis which goes beyond biology or psychology as branches of science."

Finalism as a Rock of Offence

Finalism and dynamic Teleology are closely connected, for both of them embody an attempt to go back to the Aristotelian inclusion of the final cause as an integral essential of scientific explanation, and to regard the Baconian attitude to teleology as a mistake. They solve Kant's antinomy of the teleological judgment simply by deleting the proposition, and leaving the counter-proposition. They are weakest on their practical side, for their supporters do not suggest any alterations which might be made in scientific method, although their fundamental assumptions plainly require it. The principal representative of finalism is Eugenio Rignano, and dynamic teleology has been for the most part upheld by Hans Driesch.

Rignano, in his *Qu'est-ce-que la Vie?* and his *Biological Memory* has contended that, though the mechanical concept of the universe may be perfectly satisfactory as a description of the world of physics, yet animals and plants show so much purposiveness that mechanical categories are absolutely inadequate for them. Biology, therefore, cannot be complicated physics and chemistry, but must be something *sui generis* and with its own methods and laws. "The long debate between vitalists and mechanists," he says, "in attempting to give an explanation of life, cannot lead to any conclusion unless that fundamental characteristic common to all vital phenomena of presenting a purposive, teleological, or finalistic aspect in their most typical manifestations is first thoroughly examined." The most succinct account of his views and of the exact biologist's answer to them will be found in his *Man Not a Machine*, and in the volume *Man a Machine* in the same series. The way in which they affect embryology is significant, and may be found in his chapter called "Finalism of the generative and regenerative phenomena". "Even if", he says, "the organism could be explained as a physico-chemical machine, there would still remain to be explained the most fundamental thing—how the machine constructed itself. The purposiveness of the ontogenetic development is too evident to be denied. It results from the convergence of manifold morphogenetic activities to one sole end, that is, to the formation of a marvellous functional unity, every part of which serves to maintain the life and guarantee the well-being of the whole. The embryo in its development manifests at every stage a 'harmony of composition' as Driesch calls it, which has a touch of the marvellous; parts and elements of an organ develop independently, but when they have finished their development they are found to fit together perfectly like the parts of a machine and the one so answers to the other that they unitedly form one complex organ. Thus the mouth and intestine of the sea-urchin begin their development at two points distant from each other and develop independently, but as they grow the one moves towards the other, so that when development is ended they fit together perfectly and form a single canal." This passage illustrates the line of argument found throughout Rignano, and I will not remark on it further than to draw attention to the mention of the marvellous in it, another hint, if any were needed, of that strain of misplaced "numinous instinct" which seems to be present in all biological vitalists. *Omnia exeunt in mysterium* would seem to be "a discouraging maxim" for the scientific worker. "The direction", Rignano goes on to say, "of ontogenetic development toward a predetermined end is also influenced by the fact that the embryo overcomes early disturbances which might deflect it from its course. Ontogenesis thus seems to be marshalled by some occult intelligence or entelechy in the same way that the construction of a machine and the direction of its work is presided over by the mind of the engineer."

Rignano's arguments are open to grave objection on two main grounds, first, that he regards biology as suffering more than physics from the teleology of things, and, second, that he wishes to bring the concept of purposiveness back into natural science.

The first of these is inadmissible both on philosophic and scientific grounds. Bernard Bosanquet best expresses the former attitude. He was led to his conclusions by the conviction that James Ward and other opponents of scientific naturalism had gone too far in their polemics against the mechanical theory of the universe, and had rested the case for teleology only "on the capacity of the finite consciousness for guidance and selection". This he considered a mistake. "Things are not teleological", he said, "because they are *de facto* purposed but necessary to be purposed because they are teleological....The foundations of teleology in the universe are far too deeply laid to be accounted for by, still less restricted to, the intervention of finite consciousness. Everything goes to show that such consciousness should not be regarded as the source of teleology but as itself a manifestation falling within wider manifestations of the immanent individuality of the real." Bosanquet proceeds, following out the thought of his teacher, Lotze, "The contrast, then, of mechanism with teleology, is not to be treated as if elucidated at one blow by the antithesis of purposive consciousness and the reactions of part on part. It is rooted in the very nature of totality, which is regarded from two complementary points of view, as an individual whole, and as constituted of interacting members". But Rignano's arguments are unsatisfactory also from a scientific angle, and here the objection comes from Lawrence J. Henderson, whose book *The Fitness of the Environment*, probably the most important contribution to biological thought in this century, is never referred to by Rignano. It cannot now be necessary to recount how Henderson examined the question of the finality of our present scientific knowledge, and, judging that it was considerable, went on to enquire into the properties of the elements and compounds principally associated with life. His conclusion was that living animals and plants exist in an environment just as fitted for them as they are for it, that the Darwinian concept of fitness works, indeed, both ways, and that there is a reciprocity between organism and environment so that every teleological action done by an individual organism bears upon it the image and superscription of universal teleology. Thus the conclusion of the thought of Bosanquet and Henderson was that, though teleology was a conception which it was impossible to do without, yet any limitation of it to, or special association of it with, living organisms, was inadmissible. The question remained, What has teleology to do with science?

This point has been approached best by J. W. Jenkinson with his usual clarity. "Those who uphold teleological doctrine", he said, "seem to have fallen into a confusion between two different things, the formal and the final cause. The material, efficient, and formal causes, if we mean by the last the idea of the effect in the mind of a sentient being, all precede in time the occurrence of the effect; and this kind of teleology is not, as it is asserted to be, a doctrine of final, but one of formal causes. The final cause stands for the use to which an object is to be put, the effect it will produce, the function it will perform, which obviously succeed in time the existence of the object itself. The final cause, then, cannot be taken as ever determining in time the existence of the object itself, and is therefore a conception which belongs not to science but to metaphysics. The only necessary conditions of a phenomenon ascertainable by science are those material and efficient causes which precede it." Or, as Streeter puts it, "If there is purpose in nature, we ought not to expect science to reveal it. Purpose is activity, the direction of which is determined by an end, that is, by an apprehension of quality or value. But quality cannot be measured, and therefore from its essential nature it—and along with it purpose—lies outside the sphere of science". Or, finally, to go straight to the fountain-head, "If I say", says Kant, "that I must judge according to merely mechanical laws of the possibility of all events in material nature and consequently of all forms regarded as its products, I do not therefore say: they are possible in this way alone. All that is implied is: I must always reflect on them according to the principle of the mere mechanism of nature and consequently investigate this as far as I can; because unless this lies at the basis of investigation there can be no proper knowledge of nature at all". Purposiveness, in fact, is not a conception which interlocks with quantitative treatment; that mathematical expression of relationships which is the ideal type of all science has here nothing upon which to impinge, and the pulling force, perpetually going on before, eludes and must always elude, if this analysis is correct, the advancing web of mechanical explanation.

If, then, Bacon and his successors were right in banishing the concept of teleology from scientific thought, the physico-chemical embryologist need not be alarmed by the finalism with which, according to Rignano, the whole of ontogenetic development is suffused. In fact, it is not the phenomenon, but only one way of looking at it, that is finalistic, and it is this aspect of it that must be neglected in scientific work if the gravest confusion is to be avoided. Is there need for the biologist to be any more afraid of the Drieschian entelechy (Aristotle's ἐντέλεχεια Actuality) making what might be into what is and directing from within the development of the embryo in the egg or the uterus? The word "entelechy" as used by Aristotle meant that which exists in the highest sense of the word, whether actually or potentially, e.g. the sword in the mind of the swordmaker before a single one of the necessary operations of manufacture had been begun. The entelechy therefore operated on the process in question by means of the final cause, and did not reside in the changing entity if it was dead like the sword, though it did if it was alive like the embryo. Driesch frequently says that he uses the word in a quite different sense from Aristotle, but the majority of his readers find it impossible to discover any essential point of divergence. He does at any rate make it much more precise than Aristotle, for he defines it as a non-spatial element in the living being, which at one time suspends possible action and at another time relaxes such suspension, acting in this way as the bearer of "individualising causality" and bringing the animal from potentiality into actuality.

It seems that this inherent immanent formative power has been translated by biologists of every period since Aristotle into the language of their time. Just as Driesch now tries to acclimatise it to the unfavourable environment of a post-Cartesian world, so St Gregory of Nyssa, who lived about A.D. 370, clothed it in patristic terminology, and produced a theological variety of neo-vitalism. His most important biological works, the *περὶ κατασκευῆς ἀνθρώπου*, *On the making of Man*, and *περὶ ψυχῆς*, *On the Soul*, contained such passages as these, "The thing so implanted by the male in the female is fashioned into the different varieties of limbs and interior organs, not by the importation of any other power from without, but by the power which resides in it transforming it". And elsewhere, "For just as a man when perfectly developed has a soul of a specific nature, so at the fount and origin of his life, he shows in himself that conformation of soul which is suitable for his need in its preparing for itself its peculiarly fit dwelling-place by means of the matter implanted in the maternal body, for we do not suppose it possible that the soul is adapted to a strange building, just as it is not possible that a certain seal should agree with a different impression made in wax".

Thus the soul makes its body as if it were a gem making a stamp upon some soft substance, and acting during embryogeny from within—a conception essentially like that of Driesch. We shall see later how many Renaissance authors adopted similar views, e.g. Fienus. "No unsouled thing", says Gregory, "has the power to move and to grow. Yet there is no doubt that the embryo moves and waxes big as it is fed in the body of the mother." There is nothing new about dynamic teleology; it is by no means the outcome of newly ascertained facts: it recurs from time to time in the history of biological thought because it is the natural result of an unscientific attitude.

I do not propose to discuss here the facts which originally led Driesch to the views expressed in his *Science and Philosophy of the Organism*, for they are very well known, and have been shown by J. W. Jenkinson, H. S. Jennings, H. C. Warren and A. E. Boveri, among others, to be interpretable on quite other lines. Nor shall I demonstrate by a comparison of passages from Driesch and Paracelsus how closely the conception of immanent formative force or entelechy approaches the master-archaeus of Paracelsus and the later iatrochemists such as Stahl, for Driesch has done it himself in his *History and Theory of Vitalism*. The inference from it is that the Drieschian entelechy has been and will be of no more use as a practical working hypothesis for the laboratory than the archaeus was in the past.

Driesch's dynamic teleology is open to more serious and fundamental objections. These were not obvious at the first appearance of his Gifford Lectures, but were clearly brought to light through the controversy which Jacques Loeb had with H. S. Jennings and which resulted in the publication of their respective books, *Forced Movements, Tropisms, and Animal Conduct* and *The Behaviour of the Lower Organisms*. Loeb's theory of tropisms entirely dispensed with any psychological factors, but Jennings upheld the view that they might be legitimately brought under scientific discussion, provided they were regarded as being determined as well as determining. This led him to make a new enquiry into scientific methodology, and he published his results in a valuable series of papers from 1911 to 1918. He concluded that the pursuance of laboratory work demands as its minimum of system what he called "Radical Experimental Determinism", and that there was difference of opinion as to whether this might regard conscious or unconscious mental processes as links in the chain of determinate causation. On this point Jennings and Loeb were antagonists, but both were united against Driesch, from whose writings it now appeared that psychical events might or might not affect physical events according to circumstances, and that the entelechy was subject to no general laws. Neal had maintained that the experimentally discoverable perceptual determiners in living things were insufficient to account for the effects produced in them. Jennings pointed out that, if this meant that the non-perceptual (mental) determiners acted supplementarily to the others and not instead of them, it was compatible with radical experimental determinism. But, if it was said that some of the determiners were non-perceptual and could not be known at all, then it was incompatible. Now it was just this that Driesch had been saying. "A complete knowledge", he wrote, "of all physico-chemical things and relations (including possible relations) of a given system at a time *t* would not give a complete characterisation of that system if it is a living system....Practically we may say that complete knowledge of the physico-chemical constitution of a given egg in a given state and of the behaviour following this constitution in one case, implies the same knowledge for other cases (in the same species) with great probability. But this is a probability in principle and can never be more. It would not even be a probability if we did not know the origin of a given egg in a given state, i.e. that the egg was the egg, say, of an ascidian. But to know this history or origin, is of course, already more than simply to know its physico-chemical constitution and its consequences in one case, which suffices in the realm of the inorganic. It may be that the eggs of echinoidea, fishes, and birds, are the same in all the essentials of physico-chemical constitution. Something very different happens in each case on account of the different entelechies. In spite of this we know with great probability what will happen from one case if we know that this egg comes from a bird and that from an echinoid. Therefore, practically, experimental indeterminism is not a great danger for science."

But the matter was taken up by various writers, and Lovejoy, especially, defended Driesch from the charge of interfering with the fundamental necessities of scientific thought. Jennings, however, was able to publish in reply letters from Driesch in which these implications of his position were fully admitted. "Two systems absolutely identical in every physico-chemical respect may behave differently under absolutely identical conditions if the systems are living systems. For the specificity of a certain entelechy is among the complete characteristics of a living organism and about this entelechy knowledge of physico-chemical things and relations teaches absolutely nothing." Such a basis for experimental work was generally felt to carry with it its own condemnation.

It is interesting to recall, in this connection, the vivid account given by Claude Bernard of the polemic he had with Gerdy at the Philomathic Society in Paris, for the Driesch-Lovejoy-Jennings controversy simply repeated on a larger scale the arguments of the two Parisian biologists sixty years before. "In 1859," says Claude Bernard, "I made a report to the Philomathic Society in which I discussed the experiments of Brodie and Magendie on ligature of the bile-duct, and I showed that the divergent results which the two experimentalists reached depended on the fact that one operated only on dogs and tied only the bile-duct, while the other operated only on cats, and, without suspecting it, included in his ligature both the bile-duct and a pancreatic duct. Thus I explained the difference in the results they reached and concluded that in physiology as everywhere else experiments are rigorous and give identical results wherever we operate in exactly similar conditions. *A propos* of this a member of the Society took the floor to attack my conclusions; it was Gerdy, a surgeon at the *Charité*, professor in the faculty of medicine and known through various works in surgery and physiology. 'Your anatomical explanation of these experiments', said he, 'is correct, but I cannot accept your general conclusions. You say, in fact, that the results of experiments in physiology are identical; I deny it. Your conclusion would be correct for inert nature but cannot be true for living nature. Whenever life enters into phenomena', he went on, 'conditions may be as similar as we please, the results may still be different.' To support his opinion Gerdy cited cases of individuals with the same disease, to whom he had given the same drugs with different results. He also recalled cases of like operations for the same disease, but followed by cure in one case and death in another. These differences, according to him, all depended on life itself altering the results, though the experimental conditions were the same, but this could not happen, he thought, in the phenomena of inert bodies, where life does not enter. Opposition to these ideas was prompt and general in the Philomathic Society. Everyone pointed out to Gerdy that his opinions were nothing less than a denial of biological science, but he would not give up his ideas and entrenched himself behind the word 'vitality'. He could not be made to understand that it was only a word, devoid of meaning and corresponding to nothing, and that saying that something was due to vitality amounted to calling it unknown." The only difference between Driesch and Gerdy seems to be that Driesch's arguments rested on a more solid basis of fact, and were put forward with greater eloquence.

It is worth while to study the thought of Claude Bernard more closely. Bernard was so subtle a thinker that it has always been difficult to classify him with any of the main currents of biological thought, but the following passage seems to me to sum up as well as any other the main shape of his ideas. "When a chicken develops in an egg", said he, "the formation of the animal body as a grouping of chemical elements is not what essentially distinguishes the vital force. This grouping takes place only according to laws which govern the physico-chemical properties of matter; but the guiding idea of the vital evolution is essentially of the domain of life and belongs neither to chemistry nor to physics nor to anything else. In every living germ is a creative idea which develops and exhibits itself through organisation. As long as a living being persists it remains under the influence of this same creative vital force, and death comes when it can no longer express itself; here, as everywhere, everything is derived from the idea which alone creates and guides; physico-chemical means of expression are common to all natural phenomena and remain mingled pell-mell, like the letters of the alphabet in a box, till a force goes to fetch them to express the most varied thoughts and mechanisms. This same vital idea preserves beings by reconstructing the vital parts disorganised by exercise or destroyed by accident or disease. To the physico-chemical conditions of this primal development, then, we must always refer our explanation of life, whether in the normal or pathological state." Here Bernard seems to recognise the significance of universal teleology, for he says, "here, as everywhere, everything is derived, etc.", and at the same time he lays stress on the identification of the physico-chemical aspect with the scientific aspect, going on, indeed, to say that "physiologists can only act indirectly through animal physico-chemistry, i.e. physics and chemistry worked out in the field of life, where the necessary conditions of all living organisms develop, create, and support each other according to a definite idea and obedient to rigorous determinism". It is true that elsewhere he identifies this "force that goes to arrange the letters of the alphabet" with the "mediating nature" of Hippocrates and the *archaeus faber* of van Helmont. Had he read more in Lucretius than in Aristotle, he might rather have spoken of it as a necessary outcome of the constitution of nature, a suggestion more profoundly in harmony, perhaps, with the natural bent of the scientific consciousness (cf. Bacon's remarks on Democritus in *De Augmentis Scientiarum*). But, even so, he evidently regards it as the subject-matter of metaphysics and not of science, for he says in the next paragraph, "The term 'vital properties' is only provisional because we call properties 'vital' which we have not yet been able to reduce to physico-chemical terms, though doubtless we shall succeed in that some day".

Organicism as an Occasion of Falling

By making use of the thought of Bernard, we pass by an imperceptible transition from finalism and dynamic teleology to organicism, another of the principal forms which the opposition to mechanistic biology has taken. This, like some of the other doctrines I have mentioned, has a long history behind it. The notion, "We murder to dissect", finds clear expression as early as A.D. 200, when Q. Septimius Tertullianus, of Carthage, one of the Western Fathers, spoke thus of Herophilus, the Alexandrian anatomist, "Herophilus, the physician, or rather butcher, dissected 600 persons that he might scrutinise nature; he hated man that he might gain knowledge. I know not whether he explored clearly all the internal parts of man for death itself changes them from their state when alive, and death in his hands was not simply death, *but led to error from the very process of cutting up*". No more excellent statement of the organicistic viewpoint could be devised. Sir Kenelm Digby in 1644 gave a still clearer summary of this point of view, and even in the rationalistic eighteenth century there were scientific men who objected to the use of the term machine-like as applied to animals, and insisted that the living being was an organism. Cuvier took a very definite stand on this question when he said, "All the parts of a body are interrelated, they can act only in so far as they all act together; trying to separate one from the whole means transferring it to the realm of dead substance and entirely changing its essence". But the name most familiarly associated with biological organicism in this country is that of J. S. Haldane, who has frequently set forth his views upon this subject. His attitude is so well known that it need not be described here at any length, but, in brief, he points out that the living animal is an entity with a far higher degree of internal relatedness than any non-living system, and holds that the organic cannot be understood by a study of its parts though the inorganic very possibly can. In other words, an organism is an entity whose parts lose all their characteristic properties when they are studied away from the organism itself; they fall, as it were, into meaninglessness as soon as they are abstracted from the whole of which they are parts. Consequently that kind of physiology, and *a fortiori* biophysics and biochemistry, which analyses living organisms, is insufficient as an apparatus for understanding living things and should give place to studies in which organisms are regarded intact. Moreover, it is only in the untouched organism that those wonderfully well-balanced actions are seen by which the animal or plant holds to its own niche in the economy of nature, resisting every attempt to dislodge it, provided the attempt be not so successful as to disorganise the living thing. This power of maintaining a constancy in its external and internal environment is what Haldane regards as the *deus ex machina*, the property of living things essentially inexplicable by physico-chemical hypotheses and requiring special biological language for its formulation. (For a discussion of the "inconceivability argument" in biology, see Mackenzie and Needham.) "All attempts", he says, "to trace the ultimate mechanism of life must be given up as meaningless. The aim of biology becomes a very different one—to trace in increasing detail, and with increasing clearness, the organic determination which the organic conception formulates." It is to be noted, however, that Haldane vigorously criticised Drieschian neo-vitalism, adducing against it the argument of impossibility of interference with the second law of thermo-dynamics, and the dubiousness of the theory of guidance without work done, a discussion of which on much better foundations was subsequently given by Lotka.

Haldane was not convincing in his criticism of Driesch, and there can be little doubt that Driesch's position is a perfectly tenable one, provided its supporter closes his eyes to the nature of the scientific method on the one hand, and the actual history of recent scientific progress on the other. Another side of Haldane's teaching was the view that the living animal was in some way less abstract than the world of physics; physics and biology, he thought, might some day coalesce, but it would then be found that physics would not have swallowed up biology; rather the contrary would occur and biology would swallow up physics. "The idea of life", he said, "is nearer to reality than the ideas of matter and energy, and therefore the presupposition of ideal biology is that inorganic can ultimately be resolved into organic phenomena, and that the physical world is thus only the appearance of a deeper reality which is as yet hidden from our distinct vision and can only be seen dimly with the eye of scientific faith."

There had been precursory voices of all this in the nineteenth century, as when, in spite of the discoveries of Cagniard de Latour and others that the yeast-cell played an essential part in fermentation, Justus von Liebig refused to credit them, fearing that their suggestions were a return to explanations by vital force. "Chemical actions may very well explain physiological actions, but certainly not *vice versa* ", said Moritz Traube. Claude Bernard, moreover, discussed the matter with his usual subtlety. "Physiologists", he said, "must not forget that a living being is an organism with its own individuality. Since physicists and chemists cannot take their stand outside the universe they study bodies and phenomena in themselves and separately without necessarily having to connect them with nature as a whole. Physiologists, on the contrary, find themselves outside the animal organism which they see as a whole, even when trying to get inside so as to understand the mechanism of every part. The result is that physicists and chemists reject all idea of final causes for the facts which they observe while physiologists are inclined to acknowledge an harmonious and pre-established unity in an organised body, all of whose actions are independent and mutually generative. If we break up an organism for the sake of studying its parts it is only for the sake of ease in experimental analysis, and by no means in order to conceive them separately. Indeed, when we wish to ascribe to a physiological quality its value and true significance we must always refer it to this whole and draw our final conclusions only in relation to its effect on the whole. It is doubtless because he felt this necessary interdependence among the parts of an organism that Cuvier said that experimentation was not applicable to living beings since it separated organised parts that should remain united. For the same reason vitalists proscribe experiments in medicine. These views, which have their correct side, are nevertheless false in their general outcome and have greatly hampered the progress of science." Bernard did not commit himself to an absolutely unambiguous statement as to the correct and incorrect sides of organicism, and seems to have regarded it as true only in the sense that imaginative synthesis must follow radical experimental analysis. He was therefore quite opposed to that true and keen-edged organicism represented by Cuvier and other biologists, which denied the bare utility and legitimacy of the experimental analysis, and which was not unjustly satirised by Woolf in 1927:

> You cannot demonstrate the soul
> Except upon the animal as a whole;
> Spiritual autolytic changes begin
> As soon as you push a needle through the skin.

Haldane's writings and those of his school, such as J. A. Thomson and C. G. Douglas, had extremely little effect on the direction taken by biological science in the first years of this century. As A. D. Ritchie pointed out, it is extraordinarily difficult to find out anything about living systems, unless their parts are treated in isolation, even if that be recognised as but the preliminary for imaginative synthesis, and, as many observers said, Haldane's own researches in the physiology of breathing afforded an excellent example of the usual scientific method. Biological research proceeded steadily on the usual lines, for Haldane's practical counsels could only be followed by those who were willing to abandon causal explanations in biology or to give up the hope of biology becoming an exact science.

An influence was at hand, however, which was to lessen very much, if not to destroy altogether, the attraction of Haldane's opinions for biologists. A. N. Whitehead had in his earlier works, *The Concept of Nature* and *The Principles of Natural Knowledge*, elaborated his theory of extensive abstraction, but it was not until the publication of his *Science and the Modern World* that it began to exercise any widespread effect upon scientific men other than mathematical physicists. Whitehead boldly extended the concept of the organism to cover all objects, i.e. all events, non-living as well as living. The word "inorganic" would thus cease to apply to non-living nature and all physical systems would be regarded as in a sense incomprehensible, except when regarded as wholes composed of parts owing their very existence to their share and arrangement in the whole in question. Quoting Tennyson's, "'The stars', she whispers, 'blindly run'", he says, "An electron within a living body is different from an electron outside it, by reason of the plan of the body. The electron blindly runs within or without the body, but it runs within the body in accordance with the general plan of the body and this plan includes the mental state. But this principle of modification is perfectly general throughout nature, and represents no property peculiar to living bodies".

Lloyd Morgan recognised in Whitehead's organisms his "systems of relations going together in substantial unity", which he had conceived of as stretching in degrees of ever vaster complexity from the smallest physical event to the universe itself. It was Lloyd Morgan, indeed, who pointed out first the significance of Whitehead's arguments for biological thought. He showed that the extension of organicism to cover the entire world of physics had no serious consequences for biological mechanists (who would continue to employ physico-chemical methods as before), provided that they had not adopted some form of scientific naturalism. At the same time, it could have little help for those who had insisted that the principal characteristic of living things was their organismic character, and had been led by this to propose far-reaching alterations in scientific logic or to give up the hope of causal explanation in biology. If, as it would seem, there are organisms everywhere, then the position that there are organisms nowhere turns out to be better placed than the position that living things are organisms and not other things; for, in the former case, peace can be at once secured by attention to definitions, while in the latter case the irreducible characteristic of life is not organicism, whatever else it may be. The difference between the living and the non-living becomes a quantitative one, expressible in degrees of organisation. As has been pointed out, Haldane's prophetic observations concerning the eventual meeting-place of physics and biology have perhaps at last been justified, but, if so, with a barren benefit to neo-vitalism.

Organicism and Emergence

We may now pass by another small transition, from organicism to theories of emergence. Neo-vitalism in this form practically ceases to have any claim to the name, and approaches extremely closely to neo-mechanism. The principle of emergence in its simplest form is the statement that there are levels of existence in the universe, at each of which some more complicated form of being comes into existence, containing some essence absolutely new, and which could not have been predicted, even if all the properties of the constituents of the lower order had been known. This is evidently a conception very close to that of the organism, for just as the living or non-living system, looked at from one point of view, ceases to be itself as soon as it is dismembered, so the new level of complexity, looked at from one point of view, consists of lower levels of complexity joined together in a way that could not have been foreseen, because its properties and peculiarities are not the sum of the properties and peculiarities of its constituents. But it is important to note that there are here two parentheses, namely, "looked at from one point of view", implying that there is another point of view, and though the discussion is complicated here by the doubt as to whether Whitehead intends his organisms to be taken as a metaphysical theory, or as a scientific theory, yet in Lloyd Morgan the statement is clear that the emergent point of view has always a complementary one, the resultant point of view, "the emergent web and the resultant woof" as he calls it. This is really a new and more accurate way of putting the ancient antithesis of mechanism and teleology, for the scientific method involves the concept of resultance, since it continually seeks for the predetermining causes which must be in some way uniform with their effects, while the advent of something absolutely new at each level, i.e. atom to molecule, colloidal aggregate to living cell, etc., is a speculation hardly germane to science and resembling the final cause.

We find ourselves back again, then, at the distinction between metaphysics and science, which was first seriously studied by Kant. Most of the confusion has arisen in the past through an insufficiently clear decision as to the nature of biology. Biology cannot be philosophical and scientific, emergent and resultant, indeterminate and determinate, teleological and mechanical at one and the same time.

No doubt the most powerful solvent of vitalism will turn out to be the set of changes now taking place in physics. It is as yet too early to describe very definitely the effects of these, and several modern writers on the subject are rather free in their use of the word "mysticism", but it is at any rate clear that physicists are coming to see more clearly than before the implications and the limitations of the study of the metrical aspects of the world, which is what science is. Behind these sets of numbers and quantities the background of the world is enigmatic, and Heisenberg's Principle of Indeterminacy indicates that extreme accuracy can only be obtained at a cost. Again, the abandonment of the model in physics is a highly important step, and physics seems to have reached a point at which there is no analogy in our everyday experience for the phenomena with which it is dealing, so that we cannot even picture in ordinary words what is happening. Eddington, in his great work *The Nature of the Physical World*, also shows that physics no longer speaks of the motion of every individual particle in the universe being rigidly determined but rather of the relative probabilities of its motions, i.e. the odds on them. If this funeral of Laplace's Calculator should turn out to be enduring, a good deal of difficulty may vanish from the biological sphere. But I should prefer to leave the working out of these possibilities to those better qualified than myself, and to suggest simply that if the mechanical theory of the world is being reconstructed by modern physics, there may be a widespread sapping of the force of neo-vitalistic contentions in the near future. I have often thought that neo-vitalists were thinkers whose religious sense had got into the wrong place; unable to set up commonsense watertight compartments on the one hand, or to work out a philosophy of the forms of experience on the other, they brought the numinous into biology and abused biophysics. The fundamental contention of the mechanists always was that science and the scientific method were one and that biology was complicated physics—this remains unaltered. But if physics is more and more obviously throwing off the links which bound it in the past to metaphysical materialism, and admitting itself to be the study of the metrical aspects of the world, there will be perhaps less excuse for the misplacement of the numinous, as it might be called; and the affirmation that biology must be based upon physics will cause less antagonism than in the past. In a word, the vitalists wished to introduce mysticism *at the wrong level*; the right level would seem to be, as it were, underneath the metrical abstractions of physics, i.e. outside science altogether, and if this is realised, the vitalist and the mechanist will fuse into one and the same person—a happy consummation. It is curious that at the present time, when physics is so markedly freeing itself from scientific naturalism, biologists are sometimes found to support that untenable world-view, while still farther away from the inorganic world, literary criticism tries to make itself modern by using a psychological jargon, and applying to its problems all the rigour of a materialist metaphysic.

Neo-mechanism as a Theory for Chemical Embryology

The principal philosophical obstacles to physico-chemical embryology have now been assessed and nothing remains but to outline its own theoretical basis. Anyone who was dissatisfied twenty years ago with the various forms of neo-vitalism which have already been discussed would have had no alternative but to accept the simple, though rather incredible, scientific naturalism of the preceding century, unless, indeed, he was acquainted with German philosophy, and understood the momentous consequences which flowed from the apparently technical question, "How are *a priori* synthetic judgments possible?" The varieties of neo-vitalism may perhaps be thought of, in so far as they are not modern forms of difficulties which have for many centuries perplexed philosophers, as a series of reactions against mechanistic biology insufficiently distinguished from scientific naturalism. This confusion is well seen in the earlier phases of the American discussion which led up to the symposium of 1918. It is often difficult to tell, as in the papers of Nichols; Ritter; More and Fraser Harris, whether the writer is attacking the mechanical theory of the universe regarded as an ultimate metaphysical faith or the mechanical methodology of science.

Before the eighteenth century, of course, there had always been thinkers who felt the necessity of including both teleology and its antithesis in their systems of thought. This pull in two directions accounts for those very interesting mediaeval theologians, such as Siger of Brabant and John of Jandun, who wished to acknowledge two kinds of truth, theological and philosophical (see Maywald and Gilson). A right balance had to be struck in some way between Democritus and Plotinus, and in the seventeenth century, for instance, Sir Thomas Browne wrote to his son advising him to study Lucretius yet not to read too much in him, "there being divers impieties in him". Perhaps the greatest figure in this line of descent is the Cardinal Nicholas of Cusa, who, in his book *De Docta Ignorantia* of 1440, contended that the principle of contradiction was only valid for our reason, and so foreshadowed Kant and Hegel (cf. Vansteenberghe). But the starry heavens with their infinite spaces that terrified Pascal and their mechanical order and metrical uniformity revealed by Kepler and Copernicus, on the one hand, and the moral law with its tremendous purposiveness and its theological implications, on the other hand, were never really faced at one and the same moment and taken seriously together until the time of Kant, who first subjectivated what had before struggled in the external world, and suggested that the contradictions of our thought might spring from the constitution of our own intelligence. "It has always been assumed", he said, "that all our knowledge must conform to objects. The time has now come to ask, whether better progress may not be made by supposing that objects must conform to our knowledge."

> Omnis enim longe nostris ab sensibus infra
> primorum natura jacet...

Lucretius had said, but Kant went farther, and suggested that our intelligence can help us no more than our senses in the attempt to see things as they really are.

This was the great service that Kant performed for philosophy, and in the light of it the scientific mind was relieved of the burden of having to believe finally in its own account of the world. But in the scientific controversies of the last century, Kant was forgotten, and the continual successes of the scientific method led to a naturalistic outlook, which was wholly unsatisfactory. It had been supported by T. H. Huxley, Herbert Spencer, W. K. Clifford, Tyndall, Ray Lankester, and many others: it apparently still is by Chalmers Mitchell. But, as a widely accepted attitude, it did not live long into the present century, and from such blows as James Ward's *Naturalism and Agnosticism* and Antonio Aliotta's *The Idealistic Reaction against Science*, it never recovered. Physico-chemical biologists were thus left, as it were, without visible means of support, and existed for some time on a purely pragmatic basis, devoid of any epistemological comfort. Gradually, however, a more satisfactory attitude came into being.

Karl Pearson provided an intimation of it when he remarked, "Those who say that mechanism cannot explain life are perfectly correct, but then mechanism does not explain anything. Those, on the other hand, who say that mechanism cannot describe life are going far beyond what is justifiable in the present state of our knowledge". A clear enunciation of it, informed with the charm of all his writings, was given by d'Arcy Thompson in the Aristotelian Society Symposium of 1918. "In the concepts of matter and energy", he said, "the Whole is not enshrined, mechanism is but one aspect of the world. These are the proper categories of objective science, but they are no more: the physicist is, *ipso facto*, a mechanist, but he is not by implication a materialist; nor is the biologist of necessity a materialist, even though he may study nothing but mechanism in the material fabric and bodily activities of the organism." R. S. Lillie's paper of 1927 might be taken as one of the best expositions of this point of view. "Every biologist is aware", he says, "in his non-professional moments that the possibilities of life are larger than the mechanistic view implies. This is only another way of acknowledging that the whole mechanistic conception is an incomplete, derivative, or abstract one. To regard it as philosophically final is a grave mistake." Thus Lillie remains convinced of the adequacy of physico-chemical biology, but expressly repudiates the elevation of mechanism into a metaphysic.

Lillie goes on to discuss the abstract, distorted and incomplete character of the world which is presented to us by the employment of the scientific method. "To say", he proceeds, "that life is 'nothing but' a combination of chemical reactions in a colloidal substratum is unscientific. Life may be and apparently *is* that in part but to regard any such scientific formulation as a complete and adequate representation of its total reality is simply to misconceive the structure of science." This is well said; life is indeed a "dynamic equilibrium in a polyphasic system", but also "Life is a manifest of emergent creativity", "Life is a pure flame and we live by an invisible sun within us", "Life is a sad composition, we live with death and die not in a moment", and—if you will,

Life, like a dome of many-coloured glass,
Stains the white radiance of eternity
Until death tramples it to fragments.

"The dilemma of vitalism is irresolvable", says Lillie, "so long as we regard the units, concepts, and formulae found valid in physical science not as abstractions but as primary and self-existent realities, by a combination of which all the properties of living beings as of other natural phenomena can be derived."

In a former discussion I contrasted what might be called the Democritus-Holbach-Huxley attitude in biology with the Driesch-Haldane-Russell-Rignano attitude, and concluded after examining them that both involved insuperable difficulties. Lotze was the philosopher to whom I went for help in the elaboration of a better standpoint. "The true source of the life of science", said he, "is to be found, not indeed in admitting now a fragment of one view and now a fragment of the other, but in showing how absolutely universal is the extent and at the same time how completely subordinate is the significance of the mission which mechanism has to fulfil in the structure of the world." And in another place, "We granted validity to the mechanical view in so far as concerns the examination of the relations between finite and finite and the origin and accomplishment of any reciprocal action whatever; we as decidedly denied its authority when it claimed acceptance, not as a formal instrument of investigation, but as a final theory of things". "Nowhere is mechanism", says Lotze, "the essence of the matter, but nowhere does being assume another form of finite existence except through it."

I went on to argue that, although Lotze made every effort to demonstrate how mechanism and teleology could fit together in the universe, he failed to do so convincingly, and that it was more satisfactory to make them necessary results of the a priority of our ways of thought. "We may regard", I said, "the mechanistic view of the world as a legitimate methodological distortion, capable of application to any phenomenon whatever, and possessing no value at all as a metaphysical doctrine." Mechanism, whichever of its forms (defined by C. D. Broad, M. R. Cohen and Y. H. Krikorian) turns out to be the minimum requirement of science, is, in fact, not metaphysical materialism. It is necessary to maintain it on methodological grounds, but it is pernicious to allot it any wider value. It stands, in fact, as one of the kinds of way in which the human spirit reacts to the universe in which it finds itself, and it springs, as R. G. Collingwood puts it, directly from the ultimate root of all science, the assertion of the abstract concept. "He who generalises is an idiot", said

William Blake, but that was a revelation of the poetic or the religious mind. The scientific spirit is as profound as these, but it sets out upon a different path from the very beginning and reaches in the end a country different in every way. It directs its interest from the first to the correlation of differences between phenomena rather than to individual phenomena themselves, and the impulse to classify leads inevitably to the supremacy of the mechanical cause and the mathematical formula. It stands "at diameter and sword's point" with such aphorisms as "Everything is itself and not something else" or "Nothing is ever merely anything".

R. G. Collingwood has expressed this in a memorable passage: "Mathematics, mechanics, and materialism are the three marks of all science, a triad of which none can be separated from the others, since in fact they all follow from the original act by which the scientific consciousness comes into being, namely, the assertion of the abstract concept. They are all, it may be said, products of the classificatory frame of mind, corollaries from the fact that in this frame of mind the universal and the particular are arbitrarily separated and the universal asserted in its barren and rigid self-identity. It is this barrenness and this rigidity which confer their character upon the doctrines of scientific materialism. Hence it is idle to imagine that materialism is justified in some sciences and not in others. It is idle to protest that science ought to surrender its materialistic prejudices when it finds itself face to face with a non-material object such as the soul. No object is material, in the metaphysical sense of the word, except in so far as scientific thinking conceives it so; for materiality means abstractness, subjection to the formulae of mechanical determination and mathematical calculation, and these formulae are never imposed upon any object whatever except by an arbitrary act which falsifies the object's nature. This only appears paradoxical when we fail to see the gulf which separates the common-sense materiality of a table, its sensible qualities, from its metaphysical materiality, the abstract conceptual substrate of those qualities. It is this substrate whose transcendent or abstract existence is asserted by materialism. Hence we cannot distinguish objects like tables which are really 'material' from objects in whose presence science must unlearn its materialistic habits of thought. Materialism is no more the truth in physics than in psychology, and no less. It is the truth about any object, just in so far as this object is by abstraction reducible to terms of pure mathematics; and no object is so reducible except by consciously or unconsciously shutting our eyes to everything which differentiates it from anything else. This conscious or unconscious act of abstraction is the very being of the scientific consciousness; and it is therefore no matter for pained surprise when science shows a bias towards determinism, behaviourism, and materialism generally".

By this time the general outlines of this theoretical excursus should have become clear. Embryology, to put it plainly, has been for so many years the happy hunting-ground of vitalistic and neo-vitalistic theory that the first treatise on the physico-chemical aspect of it could hardly go without some kind of theoretical introduction. "Vitalistic conceptions", said Claude Bernard in 1875, with the voice of authentic prophecy, "can no longer hover over physiology as a whole. The developmental force of the egg and the embryonic cells is the last rampart of vitalism, but in taking refuge there, it transforms itself into a metaphysical concept and snaps the last link connecting it with the physical world, and the science of physiology."

It is to be hoped that what has now been said will place in a right light the aims of physico-chemical embryology, and provide it, as it were, with its decretals. That they are not false will be the hope of every exact biologist.

Chemical embryology is now indeed at a critical point in its history. On the one hand, it links up with the morphological work of the classical embryology and the experimental work of the Entwicklungsmechanik school, while on the other hand it has affinities with genetics, a science which is every day becoming more physiological and which will more and more seek for the effects of its factors in the biochemistry of development. Goldschmidt's book, and the work of Dunn, who found a lethal gene in an inbred strain of fowl which caused a regular chick mortality at the seventeenth day of incubation, are important examples of this. Another relationship of chemical embryology is to obstetric medicine, for such problems as the toxaemias of pregnancy will not be solved by Hippocratic observation unassisted by a knowledge of the chemistry of the embryo and the placenta. The attention devoted by the Medical Research Council to such problems is an acknowledgment of this fact. Nor is veterinary physiology in a position to do without the aid of chemico-embryological researches, as is shown by the incident of the myxoedematous pig foetuses of western America in the work of Smith.

But all practical applications, how valuable soever, must give place to the increase of knowledge itself, and therefore the physico-chemical history of embryonic development, from the egg-cell to the loosing of the individual into the activity of post-natal life, is to be the theme of this present book. "The history of a man for the nine months preceding his birth", said S. T. Coleridge, "would probably be far more interesting, and contain events of far greater moment than all the three-score and ten years that follow it."

PART II

THE ORIGINS
OF
CHEMICAL EMBRYOLOGY

DII LABORIBUS OMNIA VENDUNT

Nobilissimo juveni Medico Philippo
de Glarges, amicitiae ergo libenter
Gulielmus Harveus scripsit, Anglus,
Med. Reg. et Anat. Prof. Londin.
Mai. 8 1641

From the commonplace book of
Philip de Glarges.

Not to prayse or disprayse: all did well.

William Harvey's MS. notes,
Canones Anatomiae Generalis, 6.

PRELIMINARY NOTE

It is open to anyone to say with some appearance of truth that physico-chemical embryology has no history, since the attempt to unravel the causes of embryological phenomena by physico-chemical means has only recently begun. But such a statement would betray a superficial and jejune mentality. Physico-chemical embryology has its roots in the history of embryology as a whole, and it is those roots which I shall try to uncover in what follows. It must be remembered that morphological must theoretically precede biophysical analysis, as it has actually preceded it chronologically, and to that extent physico-chemical embryology cannot be properly understood without reference to its descriptive husks, and their historical growth. Moreover, even in antiquity there are hints that the chemistry of the embryo was dimly envisaged (as in Aristotle, see p. 70). Again, that philosophical problem which we have already considered, plays a great part in the history of embryology, and as we watch the pendulum swinging from Democritus to Aristotle, back again over to Herophilus, and back once more to Galen, we almost feel as if we were spectators looking on, like Hardy's spirits, at a great drama, with the movement of which we are powerless to interfere, but knowing that the existence of exact biology depends upon which side wins. Lastly, such unmorphological questions as the respiration and nutrition of the embryo were discussed from the most ancient times, and it is surely no unduly wide interpretation of the word which leads us to include an account of these opinions under the heading of chemico-embryological history.

Nor could the present moment be more appropriate for such an historical survey. Embryology is entering a new phase: and on the threshold it is very fitting that some retrospective attention should be paid to the phases which it has already passed through. The events of the past, moreover, throw light on those of the future, and this is true not necessarily in Spengler's sense but also because the historical approach to problems actually unsolved protects them, by a kind of gentle scepticism, from too severe a subjection to doctrinaire presentations. "Die Geschichte einer Wissenschaft ist der Hort ihrer Freiheit; sie duldet ihr keine einseitige Beherrschung", said Louis Choulant in 1842. Theoretical blind alleys, such as the final cause, practical blind alleys, such as preformationism and phlogiston, are always able to remind us that we may be mistaken.

No exhaustive treatise on the history of embryology at present exists. The nearest approach to it is the very valuable memoir of Bruno Bloch with its epitome but this only covers the era of the Renaissance with thoroughness. Hertwig's account, which he printed at the beginning of his great *Handbuch der Entwicklungslehre*, does not deal very fully with any aspect of the subject before 1800, nor do the much shorter ones of Henneguy and Minot. The latter paper is interesting in that it ends with an emphasis on the need for a physico-chemical attitude in the future. The introduction to Keibel's book is much slighter, but contains some useful information. There are various monographs and papers on special points, such as Pouchet's rather untrustworthy treatment of the embryology of Aristotle, and Lones' discussion of it, which is worse. Camus' notes are still the best commentary on the *Historia Animalium*. Again, useful information on some cultural points is to be had from the treatise of Ploss & Bartels. The introductions to certain books also contain valuable information, and in this class comes Dareste's remarkable book on teratology. The bibliographies contained in v. Haller's eighth volume and in Heffter's book, are, naturally, of the greatest assistance.

These reservations made, the principal reviews of the subject are chiefly to be found in histories of science in general, such as Sarton's; histories of biological theory, such as Ràdl's; histories of obstetrics, such as v. Siebold's, Spencer's and Fasbender's; histories of gynaecology, such as McKay's; and histories of anatomy, such as Singer's and v. Töply's. Histories of medicine as a whole are numerous and helpful: I have found those of Garrison and Neuburger-Pagel most useful. Those which deal with special periods are also of assistance, such as Schrutz and Browne on Arabian, Bloch on Byzantine, and Harnack on Patristic medicine. Histories of chemistry provide no help, for ancient chemistry was so oriented towards "practical" results, such as the *lapis philosophorum* and *elixir vitae*, that the egg was only considered as a raw material for various preparations. The investigation of its change of properties during the development of the embryo did not occur to the alchemists. Detailed studies of particular subjects, such as those contained in Singer's two excellent volumes *The History and Method of Science*, may also be of some assistance. Again, there are books which give a wonderful orientation and an articulate survey of vast tracts: of these Clifford Allbutt's *Greek Medicine in Rome*, with its mass of references, is among the most valuable. And Miall's *Early Naturalists* must not be omitted, for, apart from the peculiar charm of style which marks it, it contains some singularly helpful bibliographical data. But the study of the original sources, so far as that is possible, is a duty which cannot be avoided, and in what follows I have been careful to copy down no statement from a previous review when it was possible to read the actual words of the writer himself.

This practice of going to the originals is made peculiarly necessary in a case such as the present one, when the history of a subject is to be regarded from an entirely new angle. My intention is to give here the sketch of a history of embryology consistently from the physico-chemical angle, and to show, at one and the same time, how our knowledge of the development of the embryo has come into being, and how throughout the process what we now call the physico-chemical foundations of embryonic growth have from time to time received attention, even though it was largely speculative. Since few have previously examined the history of the subject, and none from this angle, I have in many cases come upon interesting facts which have remained unknown owing to the very attitude of mind previously adopted.

Finally, I would defend the arrangement of my Sections only on the ground that it is suitable in the present state of historical knowledge. I say little about embryology in ancient China and ancient India, because on the basis of what we know there is little to say, not because it was intrinsically less interesting than the embryology of Mediterranean antiquity and the later West, though this may turn out to be the case. I do not propose a framework for historical facts in what follows; I only attempt to bring them together, and to reveal some of the relationships between them. If the traditional framework turns out to be badly constructed—and there are many signs that it may—the facts can be rearranged.

The history of single forms of scientific knowledge is in a way happier because containing more of continuity than that of civilisation as a whole. The assiduity with which men of different periods in the rise and decline of a culture pursue the different forms of human experience may, as Spengler has shown, vary much, but those forms remain fundamentally the same, even if their manifestations are profoundly changed. That science, at any rate, does maintain some sort of continuity whatever gaps there may be between the phases of its progress, is a belief agreeable with all the available facts, and one which no criticism will easily shake.

SECTION I

EMBRYOLOGY IN ANTIQUITY

1·1. Non-Hellenic Antiquity

Since biological science as a whole was little cultivated in ancient Egypt and the ancient civilisations of Babylonia, Assyria and India, the study of embryology, we may assume, was equally little pursued. Doubtless the undeveloped embryo, whether in egg or uterus, carried with it, for these persons of remote antiquity, some flavour of the obscene in the literal sense of the word. But embryology stands in a peculiar relation to the history of humanity, in that even at the most remote times children were being born, and, though the practitioners of ancient folk-medicine might confine their ideas for the most part to simple obstetrics, they yet could hardly avoid some slight speculation on the growth and formation of the embryo. Fig. 1 illustrates this level of culture. It is a painted and carved door from a house in Dutch New Guinea, taken from de Clercq's book; the original was of yellowish brown wood. The male embryo is clearly shown, but the artist evidently had a hazy conception of the umbilical cord. The line passing from the uterus to the head may or may not be merely ornamental. The movement of the foetus *in utero* played and still plays a large part in the folklore of primitive peoples, as may be read in the exhaustive treatise of Ploss & Bartels. For information concerning god-embryos in primitive religion see Briffault.

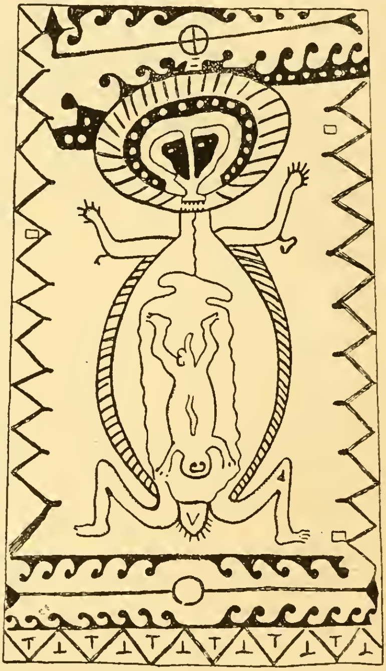


Fig. 1. Painted and carved door from New Guinea (de Clercq).

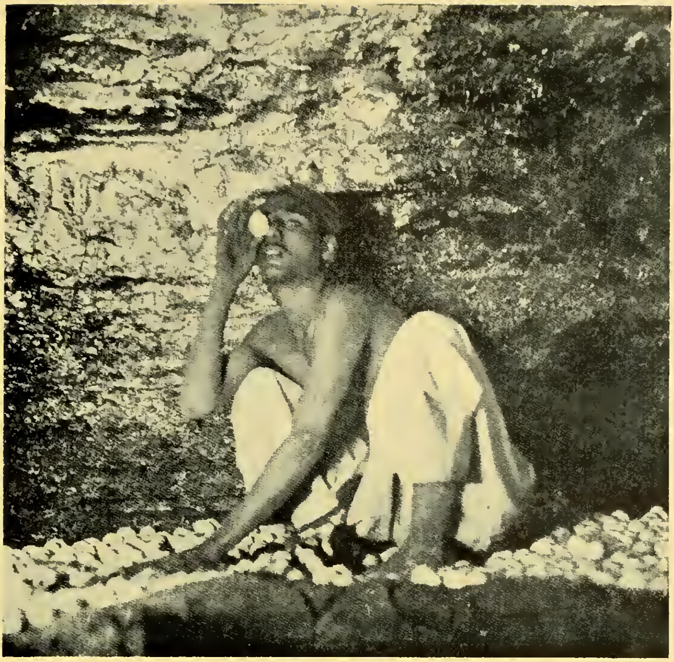
Ancient Indian embryology achieved a relatively high level. Structures such as the amniotic membrane are referred to in the

Bhagavad-Gîtâ. Susrúta believed that the embryo was formed of a mixture of semen and blood, both of which originated from chyle. In the third month commences the differentiation into the various parts of the body, legs, arms and head, in the fourth follows the distinct development of the thorax, abdomen and heart, in the sixth are developed hair, nails, bones, sinews and veins, and in the seventh month the embryo is furnished with any other things that may be necessary for it. In the eighth there is a drawing of the vital force to and from mother and embryo (is this comparable with the Hippocratic ἕλκειν? see Peck) which explains why the foetus is not yet viable. The hard parts of the body are derived from the father, the soft from the mother. Nourishment is carried on through vessels which lead chyle from mother to foetus. (For further details see Vullers.) Ancient Chinese embryology was very similar, if we may judge from Hureau de Villeneuve; Maxwell & Liu and von Martuis.

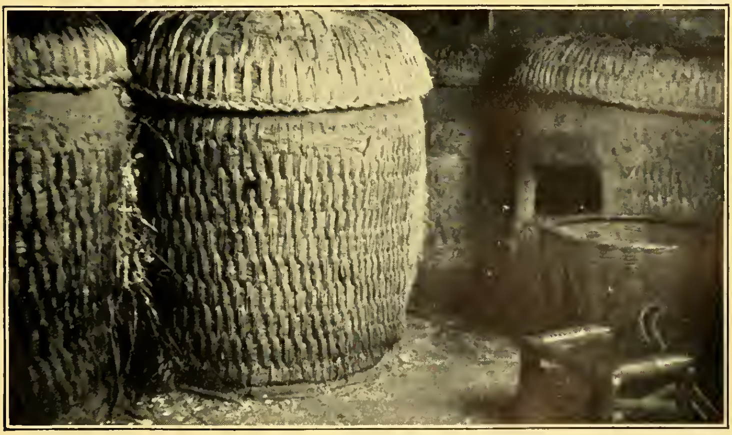
Egyptian medicine did not venture on embryological speculation, or so it would seem from the writings which have come down to us—the Ebers medical papyrus does not once mention the embryo (Brugsch). But there are points of interest as regards Egypt in this connection. The Egyptians were responsible for one of the greatest helps in systematic embryological study, namely, the discovery of the artificial incubation of the eggs of birds. The success of this process was to have so obvious an effect on embryology and the abortive attempts to bring it to completion were so frequent in the West right up to the nineteenth century A.D. that it is remarkable to find artificial incubation practised "probably", in Cadman's words, "as far back as the dawn of the Old Kingdom, about 3000 B.C." It is doubtful whether the very remote date could be supported by Egyptological evidence, for, according to Hall and Lowe, hens were not introduced into Egypt from Mesopotamia or India until the time of the eighteenth dynasty (*ca.* 1400 B.C.) when there was much intercourse with the East (cf. Queen Tiy and the Tell-el-Amarna correspondence): before then the Egyptians had only goose or duck's eggs. Artificial incubation is certainly as old as Diodorus Siculus and Pliny, for both of them refer to the practice, the latter in connection with a curious piece of ancient sympathetic magic. "Livia Augusta, the Empresse," says Pliny, "wife sometime of Nero, when she was conceived by him and went with that child (who afterwards proved to be Tiberius Caesar) being very desirous (like a yong fine lady as she was) to have a jolly boy, practised this girlish experiment to foreknow what she should have in the end; she tooke an egge, and ever carried it about her in her warme bosome; and if at any time she had occasion to lay it away, she would convey it closely out of her own warme lap into her nurses for feare it should chill. And verily this presage proved true, the egge became a cocke chicken, and she was delivered of a sonne. And hereof it may well be came the device of late, to lay egges in some warme place and to make a soft fire underneath of small straw or light chaffe to give a kinde of moderate heate; but evermore the egges must be turned with a mans or womans hand, both night and day, and so at the set time they looked for chickens and had them" (Philemon Holland's translation).

Pliny also says, "Over and besides there be some egs that will come to be birds without sitting of the henne, even by the worke of Nature onely, as a man may see the experience in the dunghills of Egypt. There goeth a pretty jeast of a notable drunkard of Syracusa, whose manner was when hee went into the Taverne to drinke to lay certaine egges in the earth, and cover them with moulde, and he would not rise nor give over bibbing untill they were hatched. To conclude, a man or a woman may hatch egges with the very heate onely of their body". This story occurs also in Aristotle.

The Emperor Hadrian—*curiositatum omnium explorator* as Tertullian calls him—writing in A.D. 130 to his brother-in-law, L. Julius Servianus, from Egypt, says, "I wish them no worse than that they should feed on their own chickens, and how foully they hatch them, I am ashamed to tell you". In the *Description de l'Egypte*, written by the members of the scientific staff of Napoleon's Egyptian expedition, and published at Paris in 1809, Rozière and Rouyer wrote on the artificial incubation of the Egyptians. They conjecture very probably that the Emperor was shocked owing to a misunderstanding shared by Aristotle, Pliny, de Pauw and Réaumur, namely, that the "gelleh" or dung was used to heat the eggs by its fermentation, and not, as is and was actually the case, by being slowly burnt in the incubation ovens. Baÿ gave an account of the ovens in modern times, but the best one is that of Lane. "The Egyptians", said Lane in 1836, "have long been famous for hatching fowls' eggs by artificial heat. This practice, though obscurely described by ancient authors, appears to have become common in ancient Egypt from an early



(A) EGYPTIAN PEASANT INCUBATOR
(from Cadman)



(B) CHINESE PEASANT INCUBATOR
(from King)

time. In Upper Egypt there are over fifty establishments, and in Lower Egypt more than a hundred. The furnace is constructed of sun-dried bricks and consists of two parallel rows of small ovens and cells for fire divided by a narrow vaulted passage, each oven being about 9 or 10 feet long, 8 feet wide and 5 or 6 feet high, and having above it a vaulted fire-cell of the same size but rather less in height. The eggs are placed upon mats or straw, one tier above another usually to the number of three tiers and the burning fuel is placed upon the floors of the fire-cells above. The entrance of the furnace is well closed. Each furnace consists of from twelve to 24 ovens and receives about 150,000 eggs during the annual period of its continuing open, one quarter or one third of which generally fail. The peasants supply the eggs and the attendants examine them and afterwards generally give one chicken for every two eggs that they have received. The general heat maintained during the process is from 100 to 103 of Fahrenheit's thermometer. The manager, having been accustomed to the art from his youth, knows from long experience the exact temperature that is required for the success of the operation without having any instrument like our thermometer to guide him. The eggs hatch after exactly the same period as in the case of natural incubation. I have not found that the fowls produced in this manner are inferior in point of flavour or in other respects to those produced from the egg in the ordinary way." The accompanying picture (Plate I A), taken from Cadman, shows the interior of a modern peasant's incubator. There is reason to believe that its construction and operation vary very little, if at all, from that of the ovens used in dynastic Egypt.

When Baÿ visited the native incubators in 1912 he took with him a flask of lime water and a thermometer. The former showed a large precipitation of calcium carbonate and the latter stood at 40° C. He was led to speculate on the value of a high CO_2 tension in the atmosphere, and concluded that it must have a beneficial effect, since the loss in the native incubator was not more than 4 per cent., while that in the oil-heated agricultural incubators of his time was as much as 40 per cent. Cadman, writing in 1921, suggests that the well-known non-sitting instinct of Egyptian poultry is an effect of the ancient practice of artificial incubation. But enough has been said of the Egyptian "Ma'mal al katakeet", or chicken factory. In spite of the remarkable opportunity thus afforded for acquiring facts in experimental embryology, no use was apparently ever made of it, though there seems to be a certain amount of traditional information current among the peasant operators, as, for example, that the "rūḥ" or life enters into the egg at the eleventh day. It would be interesting to investigate this aspect of the subject further.

In ancient China also it would appear that artificial incubation was successfully carried on in remote antiquity, if we may judge by the account given by King. Native incubation in China is carried on in wicker baskets, heated with charcoal pans (Plate I B). The attendants sleep in the incubator itself, and use the same thermometer as the Egyptians, namely, their eyelids, to which they apply the blunt end of the egg. The Egyptian success was known generally in the West in later times though it could not be imitated. "The Aegyptians", said Sir Thomas Browne, "observed a better way to hatch their Eggs in Ovens, than the Babylonians to roast them at the bottom of a sling, by swinging them round about, till heat from motion had concocted them; for that confuseth all parts without any such effect." Browne's slightly rueful tone suggests that he tried it himself. It is interesting that this quaint experiment was the cause of a controversy between Sarsi, who asserted on the authority of Suidas that it was possible, and Galileo, who thought the idea ridiculous. Modern work on the instability of albumen solutions, such as that of Harris, lends some colour to the legend. (See p. 275.)

Ancient Egypt supplies the starting-point for another and profounder train of thought which recurs constantly throughout the history of embryology, and to which I shall have to refer again more than once. This was concerned with the problem of deciding at what point the immortal constituent universally regarded as existing in living beings took up its residence in the embryo. Some fragments of ancient Indian philosophy assure us that the Vedic writers occupied themselves with this question, and according to Crawley the *Avesta* theorises upon it. But as early as 1400 B.C., i.e. during the eighteenth dynasty in Egypt, something was said regarding this, for we have extant at the present day a very beautiful hymn to the sun-god, Aton, written by no less a person than Akhnaton (Nefer-kheperu-Ra Ua-en-Ra, Amen-hetep Neter heq Uast), generally known as Amenophis IV or the "heretic" king, who abandoned the traditional worship of the Theban god Amen-Ra and established an Aton-cult, as has been described by Baikie and others. One of his hymns, which bears considerable resemblance to the one hundred and fourth psalm, runs as follows (in Breasted's translation):

(*the sun-god is addressed*)

Creator of the germ in woman,
Maker of seed in man,
Giving life to the son in the body of his mother,
Soothing him that he may not weep,
Nurse (even) in the womb.
Giver of breath to animate every one that he maketh
When he cometh forth from the womb on the day of his birth.

Thou openest his mouth in speech,
Thou suppliest his necessity.

When the fledgling in the egg chirps in the shell
Thou givest him breath therein to preserve him alive.
When thou hast brought him together
To the point of bursting out of the egg,
He cometh forth from the egg
To chirp with all his might.

He goeth about upon his two feet
When he hath come forth therefrom.

The important point here is that life = soul. At this early period there is no trace of the notions which appear later, such as the idea that embryos are not alive until the time of birth or hatching, or the idea that the soul is breathed into the embryo at some particular point in development. But in later times these considerations carried great weight, and with the rise of theology a definite stand had to be taken about them, for otherwise no ethical status could be assigned to abortion. Speculation on these matters has continued without cessation since the time of Akhnaton, reaching a climax perhaps in Christian times with Cangiamilla's *Embryologia Sacra*, and living on embedded in Roman Catholic theology up to our own era. In the last century the subject seems to have had a special fascination for Ernst Haeckel, who frequently mentioned it. But the future holds no place for the discussion of such themes, and what has been called "theological embryology" is already dead, though we may perhaps descry its successor, psychological embryology, in such researches as those of Teuscher, Cesana, y Gonzalez, Swenson and Coghill.

Ancient Greek thought shows many evidences of appreciation of the mystery of embryonic growth, as for example in the Orphic cosmogonies, which had their origin about the seventh or eighth century B.C. In these religious and legendary descriptions of the world, which have been exhaustively discussed by A. B. Cook and F. Lukas, the cosmic egg plays a large part, and has been shown to occur also in the ancient cosmogonies of Egypt, India, Persia and Phoenicia. A familiar reference to this cosmic egg, out of which all things were produced at the beginning of the world, is in Aristophanes' comedy, *The Birds*, where the owl, as leader of the Chorus, says in the Parabasis (J. T. Sheppard's translation):

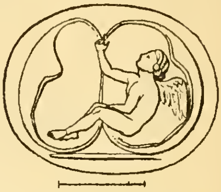


Fig. 2. Eros hatching from the cosmic egg. (A Hellenistic gem described by A. B. Cook.)

Chaos was first, and Night, and the darkness of Emptiness, gloom
tartarean, vast;
Earth was not, nor Heaven, nor Air, but only the bosom of Darkness;
and there with a stirring at last
Of wings, though the wings were of darkness too, black Night was inspired
a wind-egg to lay,
And from that, with the turn of the seasons, there sprang to the light
the Desired,
Love, and his wings were of gold, and his spirit as swift as the wind when
it blows every way.
Love moved in the Emptiness vast, Love mingled with Chaos, in spite of
the darkness of Night,
Engendering us, and he brought us at last to the light.

And perhaps another reference to the place of the egg in ancient cosmogony occurs in *The Arabian Nights*, where Aladdin's request for a roc's egg is treated as a blasphemy by the genie. Still more fantastic is the speculation of C. H. Rice (in *Psyche*, 1929!) that the world is an egg; living matter being the embryo and inorganic matter the yolk. But none of the facts which have so far been mentioned bears more than obliquely upon the main centre of interest, the study of embryology. For its direct ancestry, Greece, as might be expected, is responsible.

1·2. Hellenic Antiquity: the Pre-Socratics

The pre-Socratic philosophers nearly all seem to have had opinions upon embryological phenomena, many of which are worth referring to. These investigators of nature who lived in Greece from the eighth century onwards are only known to us through the writings of others, or in some cases in the form of fragments, for all their complete books have perished. Diels' collection of the *Fragmente der Vorsokratiker* is the most convenient source for what is left, but the assembling of their opinions has not been left to modern times, for a collection of them occurs in the writings of Plutarch of Chaeronea[1] (3rd century A.D.). It is necessary to make use of some caution in describing their views, for Aristotle, as an instance, frequently gives the most unfair versions of the views of his predecessors. The account which follows is based upon Plutarch, in Philemon Holland's translation, and Diels. Empedocles of Akragas, who lived about 444 B.C., believed that "the embryo derives its composition out of vessels that are four in number, two veins and two arteries, through which blood is brought to the embryo". He also held that the sinews are formed from a mixture of equal parts of earth and air, that the nails are water congealed, and that the bones are formed from a mixture of equal parts of water and earth. Sweat and tears, on the other hand, are made up of four parts of fire to one of water. Empedocles also had opinions about the origin of monsters and twins, and asserted that the influence of the maternal imagination upon the embryo was great so that its formation could be guided and interfered with. "Empedocles", says Plutarch, "saith that men begin to take forme after the thirtie-first day and are finished and knit in their parts within 50 daies wanting one. Asclepiades saith that the members of males because they are more hot are joynted and receive shape in the space of 26 daies, and many of them sooner, but are finished and complet in all limbes within 50 daies but females require two moneths ere they be fashioned, and fower before they come to their perfection, for that they want naturall heat. As for the parts of unreasonable creatures they come to their accomplishment sooner or later, according to the temperature of their elements." Empedocles did not consider that an embryo was fully alive. "Empedocles", says Plutarch, "denieth it to be a creature animall, howbeit that it hath life and breath within the bellie, mary the first time that it hath respiration is at the birth, namely, when the superfluous humiditie which is in such unborne fruits is retired and gone, so that the aire from without entreth into the void vessels lying open."

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[1] It is now certain that this collection is not by Plutarch himself but by an earlier compiler, Aetius (see Burnet).

Anaxagoras of Clazomenae (500–428 B.C.) may have said that the milk of mammals corresponded to the white of the fowl's egg, but that observation is also attributed to Alcmaeon of Croton. It is more certain that he spoke of a fire inside the embryo which set the parts in order as it developed, and that the head was the part to be formed first in development. This thesis was supported by Alcmaeon, and by Hippon of Samos, a Pythagorean, in the fifth century B.C., but Diogenes of Apollonia maintained about the same time that a mass of flesh was formed first, and afterwards the bones and nerves were differentiated. Plutarch remarks about this: "Alcmaeon affirmeth that the head is first made as being the seat of reason. Physicians will have the heart to be the first, wherein the veines and arteries are. Some thinke the great toe is framed first, others the navill".

The other contributions of Diogenes to this primitive embryology, were the view that the placenta is the organ of foetal nutrition, and the view that the male embryo was formed in four months but the female embryo not till five months had elapsed—a notion also found in Asclepiades and Empedocles, as we have seen. He also associated heat with the generation of little animals out of slime, and compared this with the heat of the uterus. He agreed with Empedocles that the embryo was not alive. "Diogenes saith that infants are bred within the matrice inanimate, howbeit in heat, whereupon it commeth that naturall heat, so soon as ever the infant is turned out of the mother's wombe, is drawen into the lungs." But the principal pre-Socratic embryologist was, as Zeller points out, Alcmaeon of Croton, who lived in the sixth century B.C., a disciple of Pythagoras, though apparently an independent one. He is said to have been the first man to make dissections. The fragments of Alcmaeon (who is not to be confused with Alcman, the Lacedaemonian poet) have been collected together by Wachtler; the most important are XVIII and XIX. Athenaeus in the *Deipnosophists* says, in his usual chatty way, "Now with respect to eggs Anaxagoras in his book on natural philosophy says that what is called the milk of the bird is the white which is in the eggs". This may be a wrong ascription; it may refer to Alcmaeon, for Aristotle says in his book on the generation of animals, "Nature not only places the material of the creature in the egg but also the nourishment sufficient for its growth, for since the mother-bird cannot protect the young within herself she produces the nourishment in the egg along with it. Whereas the nourishment which is called milk is produced for the young of vivipara in another part, in the breasts, Nature does this for birds in the egg. The opposite, however, is the case to what people think and what is asserted by Alcmaeon of Croton. For it is not the white that is the milk, but the yolk, for it is this that is the nourishment of the chick, whereas they think it is the white because of the similarity of the colour". Whether Aristotle was led to this conclusion because of his erroneous ideas about the part played in foetal nutrition by yolk and white respectively or whether he recognised a similarity between yolk and milk on account of their fatty nature, we cannot tell. In any case, his correction of Alcmaeon was in the right direction, and it is interesting to compare the amino-acid distribution in the casein of milk and the vitellin of yolk, as has been done by Abderhalden & Hunter (see p. 261).

Parmenides asserted a connection between male embryos and the right side of the body and between female embryos and the left side of the body—an idea which, considering its total lack of foundation, has had a very long lease of life in the world of thought. There was much controversy on the question of how foetal nutrition went on; the atomists, Democritus (born about 460 B.C.) and Epicurus (born about 342 B.C.), said that the embryo ate and drank *per os*. "Democritus and Epicurus hold", says Plutarch, "that this unperfect fruit of the wombe receiveth nourishment at the mouth; and thereupon it commeth that so soon as ever it is borne it seeketh and nuzzeleth with the mouth for the brest head or nipple of the pappe: for that within the matrice there be certain teats; yea, and mouths too, whereby they may be nourished. But Alcmaeon affirmeth that the infant within the mother's wombe, feedeth by the whole body throughout for that it sucketh to it and draweth in maner of a spunge, of all the food, that which is good for nourishment." It would appear also that Democritus believed the external form of the embryo to be developed before the internal organs were formed.

1·3. Hippocrates: the Beginning of Observation

But the foregoing fragments of speculation do not really amount to much. The first detailed and clear-cut body of embryological knowledge is associated with the name of Hippocrates, of whom nothing certain is known save that he was born probably in the forty-fifth Olympiad, about 460 B.C., that he lived on the island of Cos in the Aegean Sea, and that he acquired greater fame as a physician than any of his predecessors, if we may except the legendary names of Aesculapius, Machaon and Podalirius. It has not been believed for many centuries past that all the writings in the collection of Hippocratic books were actually set down by him, and much discussion has taken place about the authenticity of individual documents.

Most of the embryological information is contained in a section which in other respects (style, etc.) shows homogeneity. We are therefore rather interested in that unknown biological thinker who wrote the books in this class, for he could with considerable justice be referred to as the first embryologist. Littré discusses his identity, but there is no good evidence for any of the theories about it, though perhaps the most likely one is that he was Polybus, the son-in-law of Hippocrates. That the writings on generation are only slightly later than the time of Hippocrates is more or less clear from the fact that Bacchius knew of them, and actually mentions them.

For the most part the embryological knowledge of Hippocrates is concerned with obstetrical and gynaecological problems. Thus in the *Aphorisms*, ἀφορισμοί, the books on epidemics, ἐπιδημίαι, the treatise on the nature of women, περὶ γυναικείης φύσιος, the discussions of premature birth, περὶ ἐπταμήνου, the books on the diseases of women, περὶ γυναικείων, and the pamphlet on superfoetation, there are many facts recorded about the embryo, but all with obstetrical reference. There are some curious notions to be found there, such as the association of right and left breasts with twin embryos and a prognostic dependent on this.

But the three books which are most important in the history of embryology are the treatise on *Regimen*, περὶ διαίτης, the work on generation, περὶ γονῆς, and the book about the nature of the infant, περὶ φύσιος παιδίου. The two latter really form one continuous discussion, and it is not at all clear how they came to be split up into separate books. In the *Regimen* the writer expounds his fundamental physiological ideas, involving the two main constituents of all natural bodies, fire and water. Each of these is made up of three primary natures, only separable in thought and never found isolated, heat, dryness and moisture, and each of them has the power of attracting, ἕλκειν, their like, an important feature of the system. Life consists in moisture being dried up by fire and fire being wetted by moisture alternately, τροφή, the nourishment (moisture) coming into the body, is consumed by the fire so that fresh *τροφή* is in its turn required.

It is important to note that the Hippocratic school was far more akin in its general attitude to living things to modern physiology than the Aristotelian and Galenic physiology. For no considerations of final causes complicate the causal explanations of the Hippocratic school, and the author of the *περὶ διαίτης* indeed devotes seven chapters to a detailed comparison of the processes of the body (*a*) with the processes of the inorganic world both celestial and terrestrial, and (*b*) with the processes used by men in the arts and crafts, such as iron-workers, cobblers, carpenters and confectioners. These discussions present distinct mechanistic features.

He then in Section 9 sets forth his theory of the formation of the embryo. "Whatever may be the sex", he says, "which chance gives to the embryo, it is set in motion, being humid, by fire, and thus it extracts its nourishment from the food and breath introduced into the mother. First of all this attraction is the same throughout because the body is porous but by the motion and the fire it dries up and solidifies—*ὑπὸ δὲ τῆς κινήσιος καὶ τοῦ πυρὸς ξηραίνεται καὶ στερεοῦται* —as it solidifies, a dense outer crust is formed, and then the fire inside cannot any more draw in sufficient nourishment and does not expel the air because of the density of the surrounding surface. It therefore consumes the interior humidity. In this way parts naturally solid being up to a point hard and dry are not consumed to feed the fire but fortify and condense themselves the more the humidity disappears—these are called bones and nerves. The fire burns up the mixed humidity and forwards development towards the natural disposition of the body in this manner; through the solid and dry parts it cannot make permanent channels but it can do so through the soft wet parts, for these are all nourishment to it. There is also in these parts a certain dryness which the fire does not consume, and they become compacted one to another. Therefore the most interior fire, being closed round on all sides, becomes the most abundant and makes the most canals for itself (for that was the wettest part) and this is called the belly. Issuing out from thence, and finding no nourishment outside, it makes the air pipes and those for conducting and distributing food. As for the enclosed fire, it makes three circulations in the body and what were the most humid parts become the venae cavae. In the intermediate part the remainder of the water contracts and hardens forming the flesh." In this account of the formation of the embryo, which seems at first sight a little fantastic, there are several interesting things to be remarked. Firstly, there is to be noted throughout it a remarkable attempt at causal explanations and not simply morphological description. The Hippocratic writer is out to explain the development of the embryo from the very beginning on machine-like principles, no doubt unduly simplified, but related directly to the observed properties of fire and water. In this way he is the spiritual ancestor of Gassendi and Descartes. The second point of interest is that he speaks of the embryo drying up during its development, a piece of observation which anyone could make by comparing a fourth-day chick with a fourteenth-day one, and which we express to-day in graphical form (see Fig. 220). Thirdly, the ascription of the main driving force in development to fire has doubtless no direct relation to John Mayow's discovery, two thousand years later, that there is a similarity between a burning candle and a living mouse each in its bell-jar, and may mean as much or as little as Sir Thomas Browne's remark, "Life is a pure flame, and we live by an invisible sun within us". Yet the essential chemical aspect of living matter is oxidation, and the development of the embryo no less than the life of the adult is subject to this rule, so that what may have been a mere guess on the part of the Hippocratic writer, may also have been a flash of insight due to the simple observation which, after all, it was always possible to make, namely, that both fires and living things could be easily stifled.

Preformationism is perhaps foreshadowed in Section 26 of the same treatise. "Everything in the embryo is formed simultaneously. All the limbs separate themselves at the same time and so grow, none comes before or after other, but those which are naturally bigger appear before the smaller, without being formed earlier. Not all embryos form themselves in an equal time but some earlier and some later according to whether they meet with fire and food, some have everything visible in 40 days, others in 2 months, 3, or 4. They also become visible at variable times and show themselves to the light having the blend (of fire and water) which they always will have."

The work on *Generation* is equally interesting. The earlier sections deal with the differences between the male and the female seed, and the latter is identified with the vaginal secretion. Purely embryological discussion begins at Section 14, where it is stated that the embryo is nourished by maternal blood, which flows to the foetus and there coagulates, forming the embryonic flesh. The proof alleged for this is that during pregnancy the flow of menstrual blood ceases; therefore it must be used up on the way out. In Section 15 the umbilical cord is recognised as the means by which foetal respiration is carried on. Section 17 contains a fine description of development with a very interesting analogy. "The flesh", it is said, "brought together by the spirit, *τὸ πνεῦμα*, grows and divides itself into members, like going to like, dense to dense, flabby to flabby, humid to humid. The bones harden, coagulated by the heat." Then a demonstration experiment follows: "Attach a tube to an earthen vessel, introduce through it some earth, sand, and lead chips, then pour in some water and blow through the tube. First of all, everything will be mixed up, but after a certain time the lead will go to the lead, the sand to the sand, and the earth to the earth, and if the water be allowed to dry up and the vessel be broken, it will be seen that this is so. In the same way seed and flesh articulate themselves. I shall say no more on this point". Here again was an attempt at causal explanation, rather than morphological description, in complete contrast to the later work of Aristotle.

Section 22 contains a suggestive comparison between seeds of plants and embryos of animals, but the identification of stalk with umbilical cord leads to a certain confusion. Perhaps the most interesting passage of all is to be found in Section 29. "Now I shall speak", says the unknown Hippocratic embryologist, "of the characters which I promised above to discuss and which show as clearly as human intelligence can to anyone who will examine these things that the seed is in a membrane, that the umbilicus occupies the middle of it, that it alternately draws the air through itself and then expels it, and that the members are attached to the umbilicus. In a word, all the constitution of the foetus as I have described it to you, you will find from one end to the other if you will use the following proof. Take 20 eggs or more and give them to 2 or 3 hens to incubate, then each day from the second onwards till the time of hatching, take out an egg, break it, and examine it. You will find everything as I say in so far as a bird can resemble a man. He who has not made these observations before will be amazed to find an umbilicus in a bird's egg. But these things are so, and this is what I intended to say about them." We see here as clearly as possible the beginnings of systematic embryological knowledge, and from this point onwards, through Aristotle, Leonardo, Harvey and von Baer, to the current number of the *Archiv f. Entwicklungsmechanik*, the line runs as straight as Watling Street.

In Section 30 there is an important passage in which the author discusses the phenomena of birth. "I say", he says, "that it is the lack of food which leads to birth, unless any violence has been done; the proof of which is this;—the bird is formed thus from the yolk of the egg, the egg gets hot under the sitting hen and that which is inside is put into movement. Heated, that which is inside begins to have breath and draws by counter-attraction another cold breath coming from the outside air and traversing the egg, for the egg is soft enough to allow a sufficient quantity of respiration to penetrate to the contents. The bird grows inside the egg and articulates itself exactly like the child, as I have previously described. It comes from the yolk but it has its food from, and its growth in, the white. To convince oneself of this it is only necessary to observe it attentively. When there is no more food for the young one in the egg and it has nothing on which to live, it makes violent movements, searches for food, and breaks the membranes. The mother, perceiving that the embryo is vigorously moving, smashes the shell. This occurs after 20 days. It is evident that this is how things happen, for when the mother breaks the shell there is only an insignificant quantity of liquid in it. All has been consumed by the foetus. In just the same way, when the child has grown big and the mother cannot continue to provide him with enough nourishment, he becomes agitated, breaks through the membranes and incontinently passes out into the external world free from any bonds. In the same way among beasts and savage animals birth occurs at a time fixed for each species without overshooting it, for necessarily in each case there must be a point at which intra-uterine nourishment will become inadequate. Those which have least food for the foetus come quickest to birth and *vice versa*. That is all that I had to say upon this subject."

The theory underlying this passage evidently is that the main food of the fowl embryo is the white and that the yolk is there purely for constructional purposes. Had the author not been strongly attached to this erroneous view he could not have failed to notice the unabsorbed yolk-sac which still protrudes from the abdomen of the hatching chick, and if he had given this fact a little more prominence he could hardly have come to enunciate the general theory of birth which appears in the above passage. Moreover, had he been acquainted with the circulation of the maternal and foetal blood in viviparous animals, he could hardly have held that there was less food in a given amount of maternal blood at the end of development than at the beginning. At any rate, his attempted theory of birth was a worthy piece of scientific effort, and we cannot at the present moment be said to understand fully the principles governing incubation time (see p. 470).

The treatises on food and on flesh, περὶ τροφῆς and περὶ σαρκῶν, are both late additions to the Hippocratic corpus, but contain points of embryological interest. Section 30 of the former contains some remarks on embryonic respiration, and Section 3 of the latter has a theory of formation of nerves, bones, etc. by difference of composition of glutinous substances, fats, water, etc. Section 6 supports the view that the embryo is nourished *in utero* by sucking blood from the placenta, and the proof given is that its intestine contains the meconium at birth. Moreover, it is argued, if this were not so, how could the embryo know how to suck after it is born?

1·4. Aristotle

After the Hippocratic writings nothing is of importance for our subject till Aristotle. It is true that in the *Timaeus* Plato deals with natural phenomena, eclectically adopting opinions from many previous writers and welding them into a not very harmonious or logical whole. But he has hardly any observations about the development of the embryo. The four elements, earth, fire, air, and water, are, according to him, all bodies and therefore have plane surfaces which are composed of triangles. Applying this semi-atomistic hypothesis to the growth of the young animal, he says, "The frame of the entire creature when young has the triangles of each kind new and may be compared to the keel of a vessel that is just off the stocks; they are locked firmly together and yet the whole mass is soft and delicate, being freshly formed of marrow and nurtured on milk. Now when the triangles out of which meats and drinks are composed come in from without, and are comprehended in the body, being older and weaker than the triangles already there, the frame of the body gets the better of them and its newer triangles cut them up and so the animal grows great, being nourished by a multitude of similar particles." This is as near as Plato gets to embryological speculation. His description has a causal ring about it, which is in some contrast with the predominantly teleological tone of the rest of his writings; for instance, only a few pages earlier he has been speaking of the hair as having been arranged by God as "a shade in summer and a shelter in winter". It is also true that Plato may have said more about the embryo than appears in the dialogues. Plutarch mentions various speculations about sterility, and adds, "Plato directly pronounceth that the foetus is a living creature, for that it moveth and is fed within the bellie of the mother".

But all this was only the slightest prelude to the work of Plato's pupil, Aristotle. Aristotle's main embryological book was that entitled περὶ ζῴων γενέσεως, *On the Generation of Animals*, but embryological data appear in περὶ ζῴων, *The History of Animals*, περὶ ζῴων μορίων, *On the Parts of Animals*, περὶ ἀναπνοῆς, *On Respiration*, and περὶ ζῴων κινήσεως, *On the Motion of Animals*. All these were written in the last three-quarters of the fourth century B.C.

With Aristotle, general or comparative biology came into its own. That almost inexhaustible profusion of living shapes which had not attracted the attention of the earlier Ionian and Italo-Sicilian philosophers, which had been passed over silently by Socrates and Plato, intent as ever upon ethical problems, but which had been for centuries the inspiration of the vase-painters and other craftsmen (ζωγράφοι), was now for the first time exhaustively studied and reduced to some sort of order. The Hippocratic school with their "Coan classification of animals", which Burckhardt has discussed, had indeed made a beginning, but no more. It was Aristotle who was the first curator of the animal world, and this comparative outlook colours his embryology, giving it, on the whole, a morphological rather than a physiological character.

The question of Aristotle's practical achievements in embryology is interesting, and has been discussed by Ogle. There is no doubt that he diligently followed the advice of the author of the Hippocratic treatise on generation and opened fowl's eggs at different stages during their development, but he learnt much more than the unknown Hippocratic embryologist did from them. It is also clear that he dissected and examined all kinds of animal embryos, mammalian and cold-blooded. The uncertain point is whether he also dissected the human embryo. He refers in one place to an "aborted embryo", and as he was able to obtain easily all kinds of animal embryos without waiting for a case of abortion, it is likely that this was a human embryo. Ogle brings forward six or seven passages which all contain statements about human anatomy and physiology only to be explained on the assumption that he got his information from the foetus. So it is probable that his knowledge of biology was extended to man in this way, as would hardly have been the case if he had lived in later times, when the theologians of the Christian Church had come to very definite conclusions about the sanctity of foetal as well as adult life.

The περὶ ζῴων γενέσεως, the first great compendium of embryology ever written, is not a very well-arranged work. There are a multitude of repetitions, and the order is haphazard, so that long digressions from the main argument are common. The work is divided into five books, of which the second is much the most important in the history of embryology, though the first has also great interest, and the third, fourth, and fifth contain much embryological matter mixed up among points of generation and sexual physiology.

Book I begins with an introduction in which the relative significance of efficient and final causes is considered, and chapters 1 to 7 deal with the nature of maleness and femaleness, the nature and origin of semen, the manner of copulation in different animals and the forms of penis and testes found in them. Chapter 8 continues this, and describes the different forms of uterus in different animals, speaks of viviparity and oviparity, mentions the viviparous fishes (the selachians) and draws a distinction between perfect and imperfect eggs. Chapter 9 discusses the cetacea; 10, eggs in general; and 11 returns to the differences between uteri. In chapter 12 the question is raised why all uteri are internal, and why all testes are not, and in chapter 13 the relations between the urinary and the genital systems are discussed. Copulation now receives attention again, in 14 with regard to crustacea, in 15 with regard to cephalopoda, and in 16 with regard to insecta. After this point the argument lifts itself on to a more theoretical plane, and opens the question of pangenesis, into which it enters at length during the course of chapters 17 and 18, refuting eventually the widely-held view that the semen takes its origin from all the parts of the body so as to be able to reproduce in the offspring the characteristics of the parent.

The nature of semen receives a long discussion; it is decided at last that it is a true secretion, and not a homogeneous natural part (a tissue) nor a heterogeneous natural part (an organ) nor an unnatural part such as a growth, nor mere nutriment, nor yet a waste product. It is here that the theory is put forward that the semen supplies the "form" to the embryo and whatever the female produces supplies the matter fit for shaping. The obvious question has next to be answered, what is it that the female supplies? Aristotle concludes in chapters 19 and 20 that the female does not produce any semen, as earlier philosophers had held, but that the menstrual blood is the material from which the seminal fluid, in giving to it a form, will cause the complete embryo to be produced. This was not a new idea, but had already been suggested by the author of the Hippocratic περὶ γονῆς. What was quite new here, was the idea that the semen supplied or determined nothing but the form. Chapters 21 and 22 are rather confused; they contain more arguments against pangenesis, and considerations upon the contrast between the active nature of the male and the passive nature of the female. Chapter 23, which closes the first book, compares animals to divided plants, for plants in Aristotle's view fertilise themselves.

Book II opens with a magnificent chapter on the embryological classification of animals, showing Aristotle, the systematist, at his best—his classification is reproduced in Chart I. But the chapter also includes a brilliant discussion of epigenesis or preformation, fresh development or simple unfolding of pre-existent structures, an antithesis which Aristotle was the first to perceive, and the subsequent history of which is almost synonymous with the history of embryology. The question in its acutest form was not settled until the eighteenth century, but since then it has become clear that there were elements of truth in the opinion which was the less true of the two. Chapter 2 is not so important, though it has some interesting chemical analogies; it compares semen to a foam, and suggests that it was this foam, like that of the sea, which gave birth to the goddess Aphrodite[1]. But chapter 3 returns to the high level of speculation and thought found in the opening part of the book, for it deals with the degree of aliveness which the embryo has during its passage through its developmental stages. Aristotle

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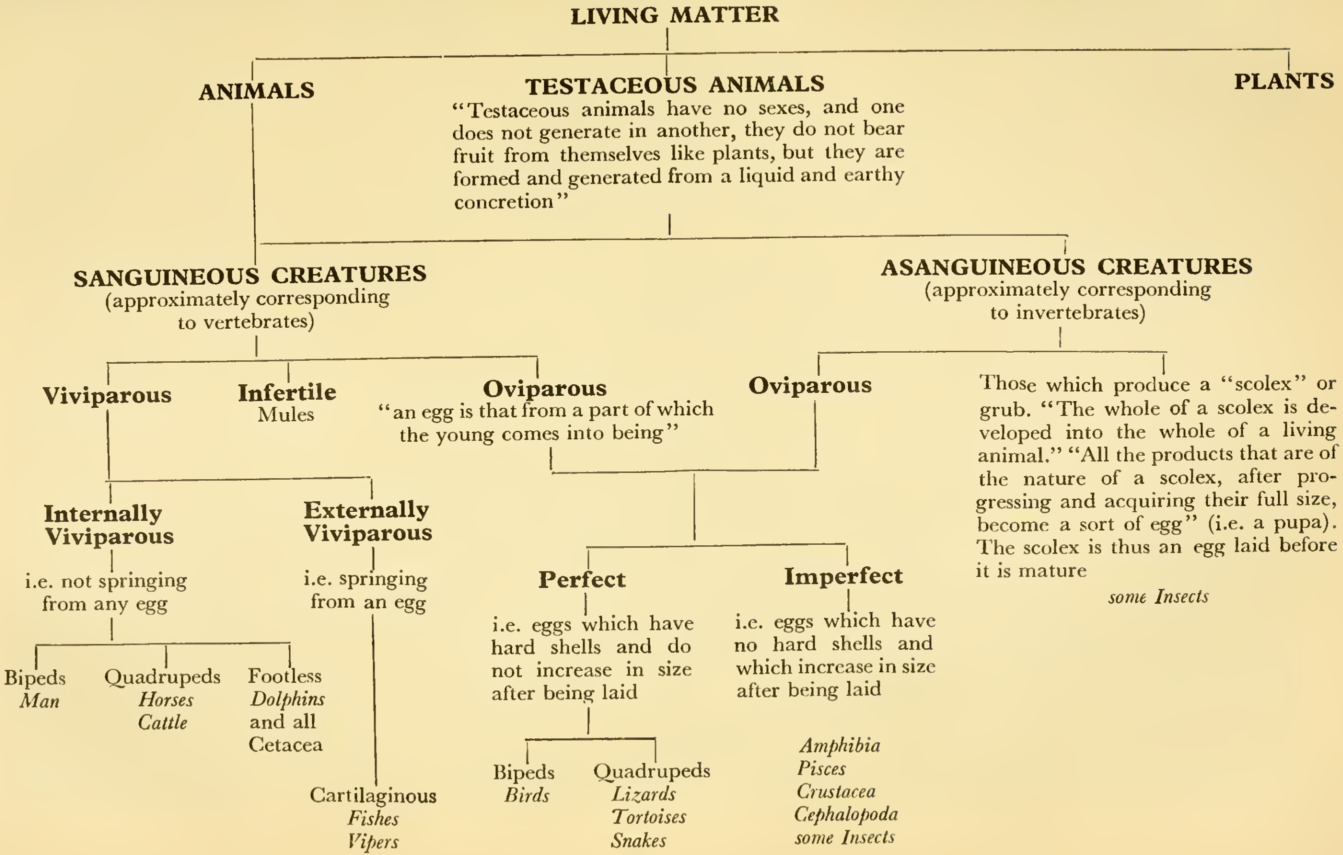
[1] To the Greeks all natural foams possessed a generative virtue, and a Zeus Aphrios was worshipped at Pherae in Thrace.

CHART I

ARISTOTLE'S CLASSIFICATION OF ANIMALS ACCORDING TO EMBRYOLOGICAL CHARACTERISTICS



LIVING MATTER

- **ANIMALS**
  - **SANGUINEOUS CREATURES** (approximately corresponding to vertebrates)
    - **Viviparous**
      - **Internally Viviparous** i.e. not springing from any egg
        - Bipeds *Man*
        - Quadrupeds *Horses* *Cattle*
        - Footless *Dolphins* and all Cetacea
      - **Externally Viviparous** i.e. springing from an egg
        - Cartilaginous *Fishes* *Vipers*
    - **Infertile** Mules
    - **Oviparous** "an egg is that from a part of which the young comes into being"
  - **ASANGUINEOUS CREATURES** (approximately corresponding to invertebrates)
    - **Oviparous**
    - Those which produce a "scolex" or grub. "The whole of a scolex is developed into the whole of a living animal." "All the products that are of the nature of a scolex, after progressing and acquiring their full size, become a sort of egg" (i.e. a pupa). The scolex is thus an egg laid before it is mature
      - *some Insects*
- **TESTACEOUS ANIMALS** "Testaceous animals have no sexes, and one does not generate in another, they do not bear fruit from themselves like plants, but they are formed and generated from a liquid and earthy concretion"
- **PLANTS**

Oviparous (sanguineous and asanguineous):

- **Perfect** i.e. eggs which have hard shells and do not increase in size after being laid
  - Bipeds *Birds*
  - Quadrupeds *Lizards* *Tortoises* *Snakes*
- **Imperfect** i.e. eggs which have no hard shells and which increase in size after being laid
  - *Amphibia* *Pisces* *Crustacea* *Cephalopoda* *some Insects*

does not here anticipate the form of the recapitulation theory, but he certainly suggests the essence of it in perfectly clear terms. This chapter has also an interest for the history of theological embryology, for its description of the entry of the various souls into the embryo was afterwards made the basis for the legal rulings concerning abortion. This chapter also discusses embryogeny as a whole, as does the succeeding one. Chapter 5 is a digression into the problem of why fertilisation is necessary by the male, but it has also some curious speculations as to what extent the hen's egg is alive, if it is infertile. The main thread is resumed in chapters 6 and 7, two very fine ones, in which embryogeny and foetal nutrition are thoroughly dealt with, but dropped again in the last section, chapter 8, which is devoted to an explanation of sterility. This ends the second book.

The third book is chiefly concerned with the application of the general embryological principles described in the previous book to the comparative field, and the fourth book contains a collection of minor items which Aristotle has not been able to speak of before.

But if the work as a whole tails off in a rather unsatisfactory manner, its merits are such that this hardly matters. The extraordinary thing is that, building on nothing but the scraps of speculation that had been made by the Ionian philosophers, and the exiguous data of the Hippocratic school, Aristotle should have produced, apparently without effort, a text-book of embryology of essentially the same type as Graham Kerr's or Balfour's. It is even very possible that Aristotle was unacquainted with any of the Coan school, for, though he often mentions Democritus, Anaxagoras, Empedocles and even Polybus, yet he never once quotes Hippocrates, and this is especially odd, for Aristotle is known to have collected a large library. Probably Hippocrates was only known to Aristotle as an eminent medical man; if this is so, Aristotle's achievements are still more wonderful.

The depth of Aristotle's insight into the generation of animals has not been surpassed by any subsequent embryologist, and, considering the width of his other interests, cannot have been equalled. At the same time, his achievements must not be over-estimated. Charles Darwin's praise of him in his letter to Ogle (which is too well known to quote) is not without all reservations true. There is something to be said for Lewes as well as Platt. Aristotle's conclusions were sometimes not warranted by the facts at his disposal, and some of his observations were quite incorrect. Moreover, he stood at the very entrance into an entirely unworked field of knowledge; he had only to examine, as it were, every animal that he could find, and set down the results of his work, for nobody had ever done it before. It was like the great days of nineteenth-century physiology, when, as the saying was, "a chance cut with a scalpel might reveal something of the first importance".

As has already been said, Aristotle regarded the menstrual blood as the material out of which the embryo was made. "That, then, the female does not contribute semen to generation", says Aristotle, "but does contribute something, and that this is the matter of the catamenia, or that which is analogous to it in bloodless animals, is clear from what has been said, and also from a general and abstract survey of the question. For there must needs be that which generates and that from which it generates, even if these be one, still they must be distinct in form and their essence must be different; and in those animals that have these powers separate in two sexes the body and nature of the active and passive sex also differ. If, then, the male stands for the effective and active, and the female, considered as female, for the passive, it follows that what the female would contribute to the semen of the male would not be semen but material for the semen to work upon. This is just what we find to be the case, for the catamenia have in their nature an affinity to the primitive matter." Thus the male dynamic element (*τὸ ἄρρεν ποιητικόν*) gives a shape to the plastic female element (*τὸ θῆλυ παθητικόν*). Aristotle was right to the extent that the menstrual flow is associated with ovulation, but as he knew nothing of the mammalian ovum, and indeed, as is shown in his embryological classification, expressly denied that there was such a thing, his main menstruation theory is wrong. Yet it was not an illegitimate deduction from the facts before him.

These views of Aristotle's about the contribution of the female to the embryo are in striking contrast with certain conceptions of a century before which were probably generally held in Greece. There is a most interesting passage relating to them in the *Eumenides* of Aeschylus, when, during the trial scene, Apollo, defending Orestes from the charge of matricide, brings forward a physiological argument. "The mother of what is called her child", Apollo is made to say, "is no parent of it, but nurse only of the young life that is sown in her (*τροφὸς δὲ κύματος νεοσπόρου*). The parent is the male, and she but a stranger, a friend, who, if fate spares his plant, preserves it till it puts forth."

There is evidence that this doctrine was of Egyptian origin, for Diodorus Siculus says, "The Egyptians hold the father alone to be the author of generation, and the mother only to provide a nidus and nourishment for the foetus". Whether this was so or not, the influence of such a doctrine must have been tremendous. We know that the conception of the female sex as playing the part of farm-land, i.e. of woman as a field in which grain was sown, was widespread in antiquity; Hartland and S. A. Cook have collected examples of it from Vedic, Egyptian and Talmudic sources. A late echo of it is to be found in Lucretius, where he refers to "Venus sowing the field of woman"—

> atque in eost Venus ut muliebria conserat arva.

Nor would resemblances between mothers and children suffice to kill this belief, for plants may differ slightly according to the soil in which they are planted. Such an idea would be the natural foundation for the practice—also widespread in antiquity—of putting captured males to death, and retaining the females as concubines. On such a theory no fear would be entertained of corrupting the race with alien blood in this way. The whole matter affords an excellent illustration of the way in which an apparently academic theory may have the most far-reaching effects on social and political events, and Aristotle, far from being remote from practical affairs as he examined his viviparous fishes and made marginal notes on his copy of Empedocles, is seen to be labouring at their very root.

The embryo, then, took its origin from the menstrual blood, on which, and in which, the seminal essence operated to produce it. But the perplexing question of the order of formation of the parts remained unsettled, though it had already been opened by earlier thinkers. What they had not done was to put the question, as it were, into the form of a motion; they had not grasped the existence of two main alternatives, one of which would have to be chosen before any further progress could be made. This is just what Aristotle did. "There is considerable difficulty", says he, "in understanding how the plant is formed out of the seed or any animal out of the semen. Everything that comes into being or is made must (1) be made out of something, (2) be made by the agency of something, and (3) must become something. Now that out of which it is made is the material; this some animals have in its first form within themselves, taking it from the female parent, as all those which are not born alive but produced as a scolex or egg; others receive it for a long time from the mother by sucking, as the young of all those which are not only externally but also internally viviparous. Such is the material out of which things come into being, but we now are enquiring not out of what the parts of an animal are made, but by what agency. Either it is something external which makes them or else it is something existing in the seminal fluid and the semen; and this must either be soul or a part of a soul, or something containing soul." Aristotle concludes that there is no external shaping influence, but only something or other contained in the embryo itself. To this extent he was wrong, for the influence of the proper physico-chemical environment on the growing embryo is as important as its physico-chemical internal constitution (later he modified his views on this). But now he goes on to deal with the main question, and says, "How then does it (the shaping influence) make the other parts? All the parts, as heart, lung, liver, eye, and all the rest, come into being either together or in succession, as is said in the verse ascribed to Orpheus, for there he says that an animal comes into being in the same way as the knitting of a net. That the former is not the fact is plain even to the senses, for some of the parts are clearly visible as already existing in the embryo while others are not; that it is not because of their being too small that they are not visible is clear, for the lung is of greater size than the heart, and yet appears later than the heart in the original development". This passage demonstrates that Aristotle had opened hen's eggs at different stages, and was well acquainted with the appearances presented there as early as the third day. He goes on to set forth a further alternative. Agreeing that a continuously new formation of parts takes place, and not merely an unfolding of parts already present in the semen or the menstrual blood, is this brought about by a chain of creations or by one original creation? In other words, does the heart come into being first, and then proceed to form the liver, and then the liver go on to form the lungs, or do they simply appear one after the other without such a creative inter-relationship? Aristotle argues against the former view on the ground that if one organ formed another, the second one would have to resemble the first in some way, which is not the case. His words on this subject cannot be condensed. "But neither can the (formative) agent be external and yet it must needs be one or other of the two. We must try then to solve this difficulty, for perhaps some one of the statements made (already) cannot be made without qualification, e.g. the statement that the parts cannot be made by what is external to the semen. For if in a certain sense they cannot, yet in another sense they can." (Thus Aristotle does some justice to the environment.) "It is possible, then, that *A* should move *B* and *B* should move *C*, that, in fact, the case should be the same as with the automatic machines shown as curiosities. For the parts of such machines while at rest have a sort of potentiality of motion in them, and when any external force puts the first of them into motion, immediately the next is moved in actuality. As, then, in these automatic machines the external force moves the parts in a certain sense (not by touching any part at the moment but by having touched one previously), in like manner also that from which the semen comes or in other words that which made the semen, sets up the movement in the embryo and makes the parts of it by having touched first something though not continuing to touch it. In a way it is the innate motion that does this, as the act of building builds a house. Plainly, then, while there is something which makes the parts, this does not exist as a definite object, nor does it exist in the semen at the first as a complete part." This notion of the setting in motion of a wound-up clock is substantially modern and underlies the physico-chemical analysis of the developing embryo. It is really striking to find Aristotle using the machine analogy in order to explain himself, for he, of all biologists, emphasised the final cause in natural operations. However, he soon returns to a more vitalistic attitude in the succeeding section. "But how is each part formed?" he says. "We must answer this by starting in the first instance from the principle that, in all products of Nature or art, a thing is made by something actually existing out of that which is potentially the same as the finished product. Now the semen is of such a nature and has in it such a principle of motion, that when the motion is ceasing each of the parts comes into being and that as a part having life or soul....And just as we should not say that an axe or other instrument or organ was made by the fire alone, so neither shall we say that foot or hand were made by fire alone....While, then, we may allow that hardness and softness, stickiness and brittleness, and whatever other qualities are found in the parts that have life and soul, may be caused by mere heat and cold yet, when we come to the principle, λόγος, in virtue of which flesh is flesh and bone is bone, that is no longer so; what makes them is the movement set up by the male parent, who is in actuality what that out of which the offspring is made is in potentiality. This is what we find in the products of art; heat and cold may make the iron soft or hard, but what makes a sword is the movement of the tools employed, this movement containing the principle of the art. For the art is the starting-point and form of the product; only it exists in something else (i.e. potentially in the mind of the artist), whereas the movement of nature exists in the product itself, issuing from another Nature (i.e. the parent) which has the form in actuality."

Thus Aristotle, evidently influenced by his doctrine of "form" and "matter", decided against preformation and pictured at one and the same time the unformed catamenia as containing a kind of clockwork mechanism which, once set in motion, would inevitably produce the finished embryo, and also as an inchoate substance on which the seminal essence should act like a swordmaker producing a sword according to the motions of a natural art. These two ideas are not completely reconciled in Aristotle, and a consideration of artificial fertilisation would have provided a test case, had he been able to know of the experiments of Delage and Loeb. For, on his second theory, butyric acid would transmute a sea-urchin's egg into butyric acid and not into a sea-urchin: while, on his first theory, the egg would make the sea-urchin irrespective of what influence it was that swung the starting-handle.

Aristotle has a good deal to say about the theory of recapitulation, as it was afterwards to be called. He thought there was no doubt that the vegetative or nutritive soul existed in the unfertilised material of the embryo, "for nobody", as he says, "would put down the unfertilised embryo as soulless or in every sense bereft of life (since both the semen and the embryo of an animal have every bit as much life as a plant) and it is productive up to a certain point....As it develops it also acquires the sensitive soul in virtue of which an animal is an animal....For first of all such embryos seem to live the life of a plant, and it is clear that we must be guided by this in speaking of the sensitive and the rational soul. For all three kinds of soul, not only the nutritive, must be possessed potentially before they are possessed actually". These passages show very clearly the line of thought contained in the recapitulation theory, as do the following. "For an animal does not become at the same time an animal and a man or a horse or any other particular animal", i.e. the more general appears first and the more particular later. "For the end is developed last, and the peculiar character of the species is the end of the generation in each individual", i.e. the embryo attains the point of being definitely not a plant before it attains that of being definitely not a mollusc but a horse or a man. Aristotle concludes that the different sorts of souls enter the embryo at different stages of development, just as the shape of the embryo gradually approximates to whatever adult shape it is destined to conform to.

Aristotle continues to discuss the central problems of embryology, but now in a way which presents features of directly physico-chemical interest. "When the material secreted by the female in the uterus has been fixed by the semen of the male (this acts in the same way as rennet acts upon milk, for rennet is a kind of milk containing vital heat, which brings into one mass and fixes the similar material, and the relation of the semen to the catamenia is the same, milk and the catamenia being of the same nature), when, I say, the more solid part comes together, the liquid is separated off from it, and as the earthy parts solidify, membranes form all round it; this is both a necessary result and for a final cause, the former because the surface of the mass must solidify on heating as well as on cooling, the latter because the foetus must not be in a liquid but separated from it." Later on, he also says, "The reason is similar to that of the growth of yeast, for yeast also grows great from a small beginning as the more solid part liquefies and the liquid is aerated. This is effected in animals by the nature of the vital heat, in yeasts by the heat of the juice contained in them".

These remarkable passages contain the first reference to enzyme action ever made in a discussion on embryology. The solidification of the outer crust is of course Hippocratic, as we have already seen. The part about the amnios is unfortunate; for the facts are exactly contrary.

"The heart is first differentiated", says Aristotle, "as is clear not only to the senses (for it is so) but on theoretical grounds. For whenever the young animal has been separated from both parents it must be able to manage itself, like a son who has set up house away from his father." This is good observation. "The heart is the principle and origin of the embryo", says Aristotle. This conception of *cor primum vivens, ultimum moriens* (a phrase never used by Aristotle himself), has henceforward a long and tortuous history, which has been described by Ebstein and others.

Aristotle goes on to describe the membranes of the mammalian foetus with its umbilical cord: "The vessels join on to the uterus like the roots of plants and through them the embryo receives its nourishment. This is why the embryo remains in the uterus"; not as Democritus thought, so that it might be moulded into the maternal shape. The embryo "straightway sends off the cord like a root to the uterus". He carefully notes, as if the conception of axial gradients was existing deep down in his mind, that the cephalic parts of the embryo are formed first. "The greater become visible before the less", he says, "even if some of them do not come into being before them. First the parts above the hypozoma" (a term more or less corresponding to "diaphragm") "are differentiated and are superior in size, the part below is both smaller and less differentiated. This happens in all animals in which exists the distinction of upper and lower, except in the insects." Aristotle gives as his explanation of this a teleological argument: "This is why the parts about the head and especially the eyes appear largest in the embryo at an early stage, while the parts below the umbilicus, as the legs, are small; for the lower parts are for the sake of the upper and are neither parts of the end, nor able to form it".

Embryonic growth is thus described by Aristotle: "The homogeneous parts (tissues) are formed by heat and cold, for some are put together and solidified by the one and some by the other.... The nutriment oozes through the blood-vessels and the passages in each of the parts, like water in unbaked pottery, and thus is formed the flesh or its analogues, being solidified by cold, which is why it is dissolved by fire. But all the particles given off which are too earthy, having but little moisture and heat, cool as the moisture evaporates along with the heat, so they become hard and earthy in character, as nails, horns, hoofs, and beaks, and therefore they are softened by fire but none of them is melted by it, while some of them, as egg-shells, are soluble in liquids. The sinews and bones are formed by the internal heat as the moisture dries, and hence the bones are insoluble by fire like pottery, for like it they have been as it were baked in an oven by the heat in the process of development.... The skin, again, is formed by the drying of the flesh, like the scum upon boiled substances; it is so formed not only because it is upon the outside, but also because what is glutinous, being unable to evaporate, remains on the surface". Here is a splendid collection of mechanical processes, but Aristotle is careful to add: "As we said, all these things must be understood to be formed in one sense of Necessity, but in another sense not of Necessity but for a Final Cause".

Concurrent growth and differentiation, the former being temporally sequent to the latter, he thus describes: "The upper half of the body, then, is first marked out in the order of development; as time goes on the lower also reaches its full size in the *sanguinea.* All the parts are first marked out in their outlines and acquire later on their colour and softness or hardness, exactly as if Nature were a painter producing a work of art, for painters too first sketch in the animal with lines and only after that put in the colours". Aristotle had some difficulty about the eyes; he noted that they were disproportionately large in early bird embryos, but he seems to have thought that they shrunk absolutely as well as relatively during further development. It takes him a great deal of ingenuity to invent a teleological explanation for this quite imaginary fact.

The food which the embryo derives from the mother, according to Aristotle, is of two distinct kinds, nutritious, formative, or creative, *τὸ θρεπτικόν*, and that which is concerned with simple increase of size, *τὸ εἰς μέγεθος ποιοῦν τὴν ἐπίδοσιν.* This distinction is difficult to understand, and, though it would be attractive to interpret the former as vitamines and the latter as fats, proteins and carbohydrates, that would probably be putting too much of a strain on our belief in Aristotle's insight. He has much to say of the placenta, and ascribes to it its correct function. He combats the idea that foetal nutrition is maintained by uterine paps, alleging against it the fact that all embryos are enclosed in membranes. He discusses birds' eggs in great detail, referring to infertile or "wind-eggs" and to the action of heat during incubation. He considered that the embryo was formed from the white exclusively, and only got its nourishment from the yolk, which was a backward step in view of what had already been said by the Hippocratic embryologist. He knew of the whiteness of the yolk when first formed in the oviduct and of the yellow colour of the layers of yolk added to it in its passage down that tube, but he held that the yellow colour was "sanguineous", and therefore hot, while the white was cold. He held also that the bird embryo always developed at the pointed end; no doubt, as Platt has suggested, Aristotle belonged to Swift's class of "Little-endians", and must have always opened them at that end, in which case he would find the embryo there, for the yolk always swims embryo uppermost. He knew also that the yolk liquefied during the first week of development, and that it grew larger, but he did not guess the right reasons of these phenomena. He knew the arrangements for embryonic development in the dolphins and ovoviviparous sharks. He takes a strong line over spontaneous generation; "nothing", he says, "comes into being by putrefying, but by concocting". And so in many other passages where detailed observation is joined with acute reasoning. So far only the treatise on the generation of animals has been under consideration. But in the περὶ ζῴων, also, there are many embryological data, and it is strange that those detailed observations upon the developing fowl embryo, which demonstrate more than anything else Aristotle's wonderful powers of observation, are not contained at all in the *Generation of Animals*, but in the *History of Animals*. He takes animals one by one in order, and in each case deals with their generative peculiarities, such as their mode of hatching, their incubation period, their fertility, etc. For instance, he correctly relates how cartilaginous fish embryos possess a yolk-sac like bird embryos, but no allantois. In his account of the fowl he is unusually precise.

Most of the sixth book is occupied with the account of the generation of birds and fishes, and the seventh treats also very fully of that of man. But in both cases it is a description that is given; more theoretical considerations are left to the book on generation, and for this reason the latter work is the more interesting. From a general point of view the *History of Animals* has a more wonderful wealth of material in it than the book on generation, but, at the same time, it also indulges in much more extravagant stories, such as those of the "kindly and gentle dolphin" and the equine Oedipus, and to that extent the austerity of the book on generation charms us more.

Other treatises also mention embryology. The περὶ ζῴων μορίων, *On the Parts of Animals*, has a passage in which the appearance of the third-day chick embryo is described, and refers to observations on the lack of pigment and of distinct medullary canals in bones in foetal life. The small work entitled περὶ ἀναπνοῆς, *On Respiration*, also refers to the heart as the first organ to be formed, and so as the seat of the soul. But these minor sources contribute little to the progress of the science, and it is upon the great work *On the Generation of Animals* that Aristotle's well-deserved fame as an embryologist will always rest.

If I have devoted a very large space to an account of Aristotle's contributions to embryology, it is, firstly, because they are actually greater in number than those of any other individual embryologist, and secondly, because they had so profound an influence upon the following twenty centuries. Embryology from the third century B.C. to the seventeenth century A.D. is meaningless unless it is studied in the light of Aristotle.

His outstanding contributions to embryology may be put in the following way:

1. He carried to their logical conclusion the principles of the observation of facts suggested by the unknown Hippocratic embryologist, and added to them a discipline of classification and correlation of facts which gave embryology a quite new coherence.
2. He introduced the comparative method into embryology, and by studying a multitude of living forms was able to lay the foundation for future science of the various ways in which embryonic growth can take place. Thus he knew of oviparity, ovoviviparity, and viviparity, and one of his distinctions is substantially the same as that known to modern embryology between holoblastic and meroblastic yolks.
3. He distinguished between primary and secondary sexual characteristics.
4. He pushed back the origin of sex-determination to the very beginning of embryonic development.
5. He associated regeneration phenomena with the embryonic state.
6. He realised that the previous speculations on the formation of the embryo could be absorbed into the definite antithesis of preformation and epigenesis, and he decided that the latter alternative was the true one.
7. He put forward a conception of the unfertilised egg as a complicated machine, the wheels of which would move and perform their appointed function in due course when once the master-lever had been released.

8. He foreshadowed the theory of recapitulation in his speculations on the order in which the souls came to inhabit the embryo during its growth, and in his observation that universal characteristics precede particular characteristics in embryogeny.
9. He foreshadowed the theory of axial gradients by his observations on the greater and more rapid development of the cephalic end in the embryo.
10. He allotted the correct functions to the placenta and the umbilical cord.
11. He gave a description of embryonic development involving comparison with the action of rennet and yeast, foreshadowing thus our knowledge of organic catalysts in embryogeny.

But there was another side to the picture. Aristotle made three big mistakes, and here I do not refer to any matters of detail, in which it would not have been humanly possible to be more than very often right, but rather to general notions, such as the eleven correct ones.

They were as follows:

1. He was incorrect in his view that the male supplies nothing tangible to the female in the process of fertilisation. To say that the semen gave the "form" to the inchoate "matter" of the menstrual blood was equivalent to saying that the seminal fluid carried nothing in it but simply an immaterial breath along with it. Aristotle did not envisage the existence of spermatozoa.
2. He was entirely wrong in his teaching about the *scolex*. The caterpillar is not, as he supposed, an egg laid too soon, but has already passed through the embryonic state.
3. He was misled by some observations on castrated animals and so did not ascribe to the testis its true function.

Such mistakes as these, however, were not nearly so important as the solid ground gained by his correct answers. They were always open to experimental test, even though the authority of his name precluded it until the Renaissance. But there was one aspect of his embryological work which was to exercise an unfortunate influence on the subsequent progress of the science, namely, his insistence on teleological explanations. He was always seeking for final as well as efficient causes. "The ancient Nature-philosophers did not see that the causes were numerous; they only saw the material and efficient causes and did not distinguish even these, while they made no enquiry at all into the formal and final causes." "Democritus", he says, "neglecting the final cause, reduces to Necessity all the operations of Nature. Now they are necessary it is true, but yet they are also for a final cause, and for the sake of what is best in each case."

Now in Aristotle this was all to the good. A metaphysician as well as a scientific worker, he was able to use the concept of purposiveness as a heuristic aid, but he never *rested* upon it. The trouble was that he introduced it into the discussion at all. It is an interesting speculation to consider what would have happened if the first great biologist had not brought final causes into his teaching; perhaps the subsequent history of biology, and science as a whole, would have been very different. For final causes irresistibly led to the theological blank alleys into which men's thoughts were ushered and there left to grope till the end of the Middle Ages.

Perhaps Aristotle would not have made so many great discoveries if he had been more of a Democritus. For teleology is, like other varieties of common sense, useful from time to time; e.g. Harvey told Boyle that he was led to certain important considerations by meditating upon the final cause of the valves in the veins; and every biologist acts in the same way at the present time. But the important thing is not to give the last word to teleology. And those attractive shady places which Aristotle, guided by his genius, quickly passed through on his perpetual journeys into the hot sunlight of research and speculation were so many traps for those who followed him. He himself knew how to change rapidly from metaphysician into physicist and back again, how to bow politely to the final cause and press on with the dissection; but the later Peripatetics had no knowledge of this art, nor had the Patristic Doctors, nor the mediaeval Aristotelians; who all remained sleeping quietly in the shade of the will of God. He knew very well from the sea (to use Bacon's metaphor at last) the look of the Circë country of teleology, but he never visited it for long at a time, being an authentic Odysseus, unlike so many later heads, who, following the example of Plato, "anchored upon that shore" and, dropping their hooks to the sound of plain-song, there rode, never to hoist sail again.

1·5. The Hellenistic Age

Aristotle died in 322 B.C. From that year until 1534, the date of the birth of Volcher Coiter, first in time of the Renaissance embryologists, embryology has very little history.

The founder of the stoical philosophy, Zeno of Citium, was born some twenty years before the death of Aristotle. "Pious and magnanimous as Stoicism was in the field of conduct", says Allbutt, "creating or nourishing that elevation of mind which distinguished the nobler Roman of the Empire, yet in Rome, as in England, its natural science was of no account. The spirit of it was indeed rather alien than akin to science. The mind of the Porch which called itself 'practical' was reluctant to all 'speculation', natural science included." The Stoics regarded the four qualities of cold, hot, wet, and dry, as ultimate, instead of the earth, fire, air, and water of the Peripatetics and their predecessors. Plutarch, in his summary of philosophic opinions already mentioned, has some passages relating to their views on the development of the embryo. "The Stoicks say", he relates, "that the foetus is fed by the fecundine and navell; whereupon it is that midwives presently knit up and tie the navell string fast, but open the infants mouth, to the end that it be acquainted with another kind of nourishment." And elsewhere, "The Stoicks say that it is a part of the wombe and not an animall by itselfe. For like as fruits be parts of trees, which when they be ripe do fall, even so it is with an infant in the mother's wombe....The Stoicks are of opinion that the most parts are formed all at once; but Aristotle saith the backbone and loines are first framed like as the keele in a ship." But to which of Zeno's successors, Cleanthes, Chrysippus, Crates or the rest, these sayings are to be attributed, is not known.

The Epicureans also had opinions on these subjects. They thought that the foetus *in utero* was fed by the amniotic liquid or the blood, and they also believed, in contradistinction to the Peripatetics, that both male and female supplied seed in generation, as is shown by the lines of Lucretius:

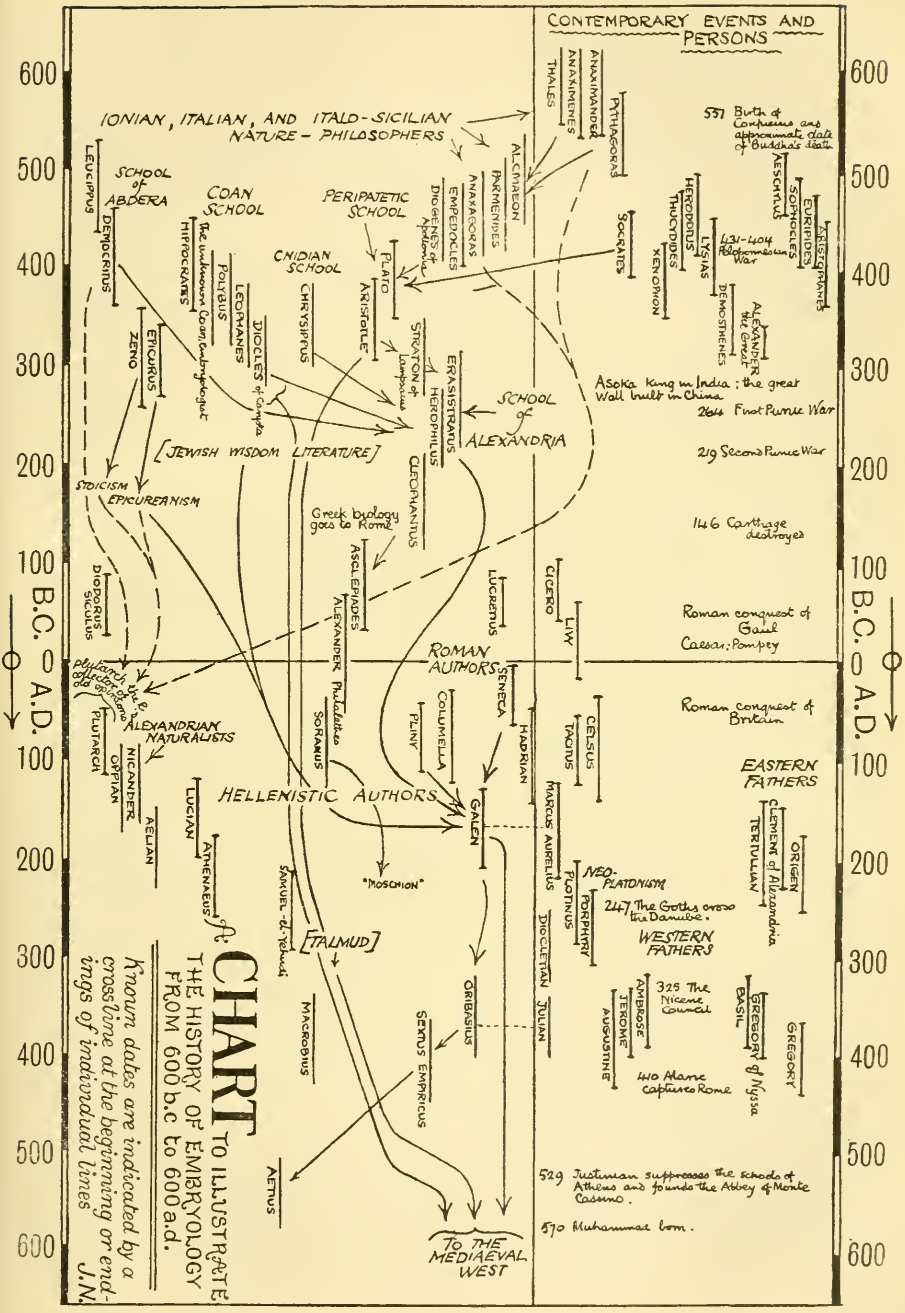
usque adeo magni refert, ut semina possint
seminibus commisceri genitaliter apta
crassaque conveniant liquidis et liquida crasso.

But much more important than the teaching of these philosophers was the rise of what might be called the scientific faculty of the great

University of Alexandria. That seat of learning, perhaps the most glorious, after Athens, of any in antiquity, and greater than its contemporary rival Pergamos, was important because all the traditions of earlier times were united in it like a bundle of strands coming together to form a rope. Democritean atomism, Peripatetic science and metaphysics, Coan biology, Coan and Cnidian medicine, above all, Athenian mathematics and astronomy, all were gathered in the *μουσεῖον* of Alexandria under the benevolent dynasty of the Ptolemies. The link between the Alexandrian biologists and the school of Aristotle was Straton of Lampsacus, who, though apparently not making any contribution to embryology himself, must have brought the knowledge of generation gained by Aristotle to Alexandria as he sailed south across the Mediterranean to be the tutor of Ptolemy Philadelphus. The link between Cos and Alexandria was Diocles of Carystus, who was the last of the Hippocratic school and also a pupil of Philistion of Locri. Diocles has a certain importance in the history of embryology; for Oribasius refers to him as the discoverer of the *punctum saliens* in the mammalian embryo, "on the ninth day a few points of blood, on the eighteenth beating of the heart, on the twenty-seventh traces of the spinal cord and head". He thus showed that the early development of chick and mammal was very alike. Plutarch also tells us that he occupied himself with the question of sterility. He described the human placenta, as well as embryos of twenty-seven and forty days, and he held that both male and female contribute seed in generation. Cnidian medicine influenced Alexandria through Chrysippus of Cnidus—not the Stoic—whose embryological doctrine seems to have been that the embryo had only a vegetative soul until birth or hatching.

All these influences were fruitful, for they produced the two greatest physiologists of ancient times, Herophilus of Chalcedon and Erasistratus of Chios. These two, who were contemporaries during the third century B.C., experimented much and wrote voluminously, but all except fragments of their writings have been lost, and can now only be pieced together out of the books of Galen, as has been done by Dobson. Allbutt has well described the differences between them, such as the predilection of Herophilus for the humoral pathology and pharmacy, and the greater interest taken by Erasistratus in atomistic speculations. "Herophilus", says Plutarch, "leaveth to unborne babes a mooving naturall, but not a respiration, of which

CHART II



A CHART TO ILLUSTRATE THE HISTORY OF EMBRYOLOGY FROM 600 b.c to 600 a.d.

Known dates are indicated by a crossline at the beginning or endings of individual lines

J.N.

motion the sinewes be the instrumentall cause, but afterwards they become perfect living animall creatures, when being come forth of the wombe, they take in breath from the aire."

Herophilus described the ovaries and the Fallopian tubes, but did not advance further than Aristotle towards correct sexual physiology in this respect. We gather that he made many dissections of embryos from the testimony of Tertullian, though this may not be worth much. Moreover, he called the outer membrane of the brain, chorion, after the membranes which surround the embryo. He gave a correct description of the umbilical cord, except that he assigned to it four vessels instead of three, carrying blood and breath to the embryo. The veins, he thought, communicated with the venae cavae, and the arteries with the great artery running along the spine. Herophilus also occupied himself much with obstetrical matters, and wrote a treatise on them, *μαιωτικόν*. Together with Erasistratus he denied that there were any diseases special to women other than those attendant on their special sexual functions, but the greatest contribution which he made to biology was the association of the brain with the intellect[1], for even Aristotle had made the heart the seat of the mental individual.

Erasistratus did not study embryology as much as did Herophilus, but a passage in Galen throws an interesting light on his notions of embryonic growth. "The heart", says Galen, "is no larger at first than a millet seed, or, if you like, a bean. Ask yourself how it could grow large otherwise than by being distended and receiving nutriment throughout its whole extent, just as we have shown above that the seed is nourished. But even this is unknown to Erasistratus, who makes so much of Nature's Art. He supposes that animals grow just like a sieve, a rope, a bag, or a basket, each of which grows by the addition to it of materials similar to those out of which it began to be made." This is only one instance out of many in which Galen, the teleologist, finds fault with Erasistratus, the mechanistic philosopher.

During the period when the biological school of Alexandria was at its height, that city became an important Jewish centre. Two centuries later it was to produce Philo, but now the Alexandrian Jews were writing that part of the modern Bible known as the Wisdom Literature. In books such as the Wisdom of Solomon, Ecclesiasticus, Proverbs, etc., the typical Hellenic exclusion of the action of gods in natural phenomena is clearly to be seen. There are two passages of embryological importance. Firstly, in the book of Job (x, 9–11), Job is made to say, "Remember, I beseech thee, that thou hast fashioned me as clay; and wilt thou bring me into dust again? Hast thou not poured me out as milk, and curdled me like cheese? Thou hast clothed me with skin and flesh, and knit me together with bones and sinews". This comparison of embryogeny with the making of cheese is interesting in view of the fact that precisely the same comparison occurs in Aristotle's book *On the Generation of Animals*, as we have already seen. Still more extraordinary, the only other embryological reference in the Wisdom Literature, which occurs in the Wisdom of Solomon (vii, 2), also copies an Aristotelian theory, namely, that the embryo is formed from (menstrual) blood. There the speaker says, "In the womb of a mother was I moulded into flesh in the time of ten months, being compacted in blood of the seed of man and the pleasure that came with sleep". Perhaps it is no coincidence that both these citations can be referred back to Aristotle, and, in the second case, even to Hippocrates; perhaps the Alexandrian Jews of the third century B.C. were studying Aristotle as attentively as Philo Judaeus studied Plato a couple of hundred years later.

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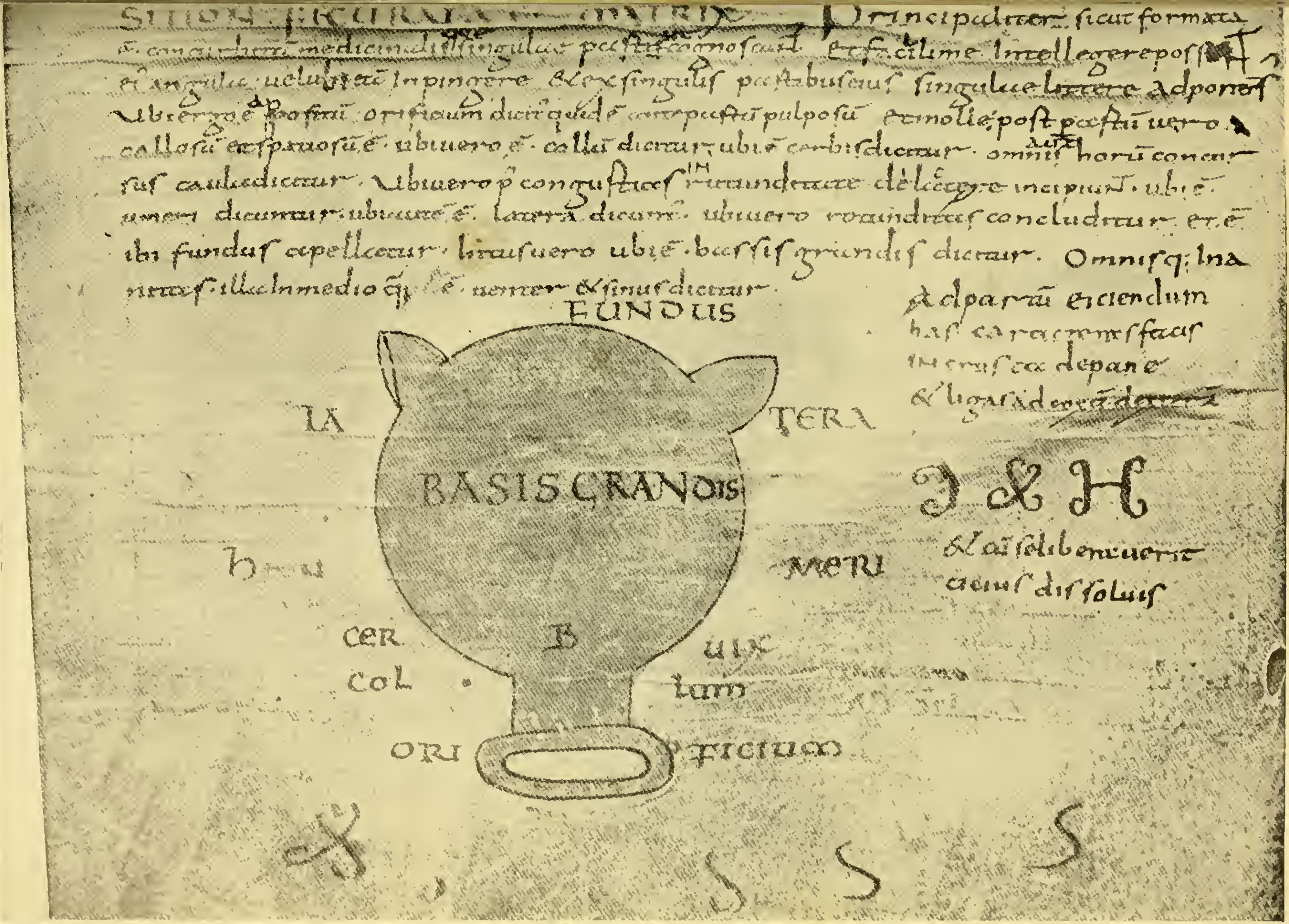
[1] This was not absolutely new: Alcmaeon had held the same view (see Burnet).

The Alexandrian school was directly responsible for the introduction of Greek medicine and biology into Rome, through the physician Cleophantus, who seems to have been particularly interested in gynaecology. At the end of the second century B.C. and the beginning of the first, Rome received the first and greatest of her Greek physicians, Asclepiades of Parion, who brought atomism with him. He was thus the link between Epicurus and the methodistic school of physicians, and may have been a potent influence upon Lucretius. Again, Alexander Philalethes provides the link between Cleophantus and Soranus. Soranus lived in Rome from about A.D. 30 till just after the end of the first century, and so twenty years before the birth of Galen.

Of all the ancient writers on embryology, Soranus is the one whose works were in later times most widely appropriated, mutilated, furbished up, quoted from rightly and wrongly, and generally upset. Allbutt, Barbour and Singer give accounts of the way in which this process went on, and the whole question has given rise to a considerable literature. (See Lachs, Ilberg, Sudhov, etc.) It lasted right into the Middle Ages, and was particularly vehement in the case of the treatise on gynaecology, περὶ γυναικείων παθῶν. This was translated into Latin under the name of Moschion, then back into Greek and finally back into Latin again. It is largely obstetrical, but it shows an advanced knowledge of embryology, and especially an accurate idea of the anatomy of the uterus. (See Plate II.)

The other writers of this period are unimportant embryologically. Among the Greeks, Aelian wrote a *De natura animalium*, in which he spoke of eggs, but without adding anything to our knowledge of them; Nicander in his *Theriaca* refers to mammalian embryos, and alleges that they breathe and eat through the umbilical cord; and Oppian has a few unsystematic remarks about the embryos of various animals. Junius Columella's work on husbandry contains two chapters on eggs, but he was not much interested in the theoretical aspect of development. In Aulus Gellius we have the cheese analogy appearing in conjunction with obscurantist views about the powers of the number seven. It is not generally known that a clear statement of the preformationist or "Entfaltung" theory of embryogeny occurs in Seneca's *Quaestiones naturales*, where there is the following passage: "In the seed are enclosed all the parts of the body of the man that shall be formed. The infant that is borne in his mother's wombe hath the rootes of the beard and hair that he shall weare one day. In this little masse likewise are all the lineaments of the bodie and all that which Posterity shall discover in him". Perhaps this notion was derived by Seneca from the Homoeomereity of Anaxagoras, for a discussion of which in relation to embryology, see Cornford. "Hair cannot come out of not-hair, nor flesh out of not-flesh", said Anaxagoras.

The *Natural History* of Pliny, that "voluminous, industrious, unphilosophical, gullible, unsystematic old gossip", as Singer justly calls him, contains little of embryological importance, although he devotes many sections to eggs, and what there is comes straight from the fountain-head, Aristotle. As, for example, "All egs have within them in the mids of the yolk, a certain drop, as it were of bloud, which some thinke to be the heart of the chicken, imagining that, to be the first that in everie bodie is formed and made; and certainlie a man shall see it within the verie egge to pant and leape. As for the chick, it taketh the corporall substance, and the bodie of it is made of the white waterish liquor in the egge, the yellow yolke


FUNDUS
LA TERA
BASIS GRANDIS
B
CER UIX
COL LUM
ORI FICIUM
MERI
I & H

THE OLDEST KNOWN DRAWING OF THE UTERUS. It occurs in a 9th century MS. (the Brussels Moschion codex) of Soranus' work on gynaecology.

serves for nourishment; whiles the chick is unhatched and within the egge, the head is bigger than all the bodie besides; and the eies that be compact and thrust together be more than the verie head. As the chick within growes bigger, the white turneth into the middest, and is enclosed within the yolke. By the 20 day (if the eggs be stirred) ye shall heare the chick to peepe within the verie shell; from that time forward it beginneth to plume and gather feathers; and in this manner it lies within the shell, the head resting on the right foot, and the same head under the right wing, and so the yolke by little and little decreaseth and faileth". But the best way to illustrate Pliny's embryology is to copy out some of his index, as follows:

The Table to the first Tome of Plinies Naturall Historie.

Egs diverse in colour	298
Egs of birds of 2 colours within the shell	ibid.
Egs of fishes of 1 colour	ibid.
Egs of birds, serpents, and fishes, how they differ	ibid.
Egs best for an hen to sit upon	299
Egs hatched without a bird, onely by a kind heat	ibid.
Egs how they be marred under an hen	ibid.
wind-egs, called Hypenemia	300
how they be engendred	301
wind-egs, Zephyria	ibid.
Egs drawne through a ring	ibid.
Egs how they be best kept	ibid.

The Table to the second Tome of Plinies Naturall Historie.

Egs of hens and their medicinable properties	351
yolke of hens egs, in what cases it is medicinable	352
Egs all yolke, and without white, be called Schista	ibid.
skinne of an Hens egge-shell, good in Physicke	ibid.
Hens Eggeshell reduced unto ashes, for what it serveth	ibid.
the wonderfull nature of Hens Eggeshels	ibid.
Hens Egges, all whole as they be, what they are good for	353
the commendations of Hens Egges, as a meat most medicinable	ibid.
Hens Egge, a proper nourishment for sicke folks, and may go for meat and drinke both	ibid.
Egge-shels, how they may be made tender and pliable	ibid.
white of an Egge resisteth fire	ibid.
of Geese Egges a discourse	354
the serpents egge, which the Latines call Anguinum, what it is, and how engendred	355

This last item exhibits Pliny at his worst. It is worth quoting, apart from its intrinsic value, for it shows to what depths embryological knowledge descended within four hundred years after Aristotle collected his specimens on the shores of the lagoon of Pyrrha, and talked with the fishermen of Mitylene. "I will not overpasse one kind of eggs besides, which is in great name and request in France, and whereof the Greeke authors have not written a word; and this is the serpents egg, which the Latins call Anguinum. For in Summer time yerely, you shall see an infinit number of snakes gather round together into an heape, entangled and enwrapped one within another so artificially, as I am not able to expresse the manner thereof; by the means therefore, of the froth or salivation which they yeeld from their mouths, and the humour that commeth from their bodies, there is engendred the egg aforesaid. The priests of France, called Druidae[1], are of opinion, and so they deliver it, that these serpents when they have thus engendred this egg do cast it up on high into the aire by the force of their hissing, which being observed, there must be one ready to catch and receive it in the fall again (before it touch the ground) within the lappet of a coat of arms or souldiours cassocks. They affirme also that the party who carrieth this egg away, had need to be wel mounted upon a good horse and to ride away upon the spur, for that the foresaid serpents will pursue him still, and never give over until they meet with some great river betweene him and them, that may cut off and intercept their chace. They ad moreover and say that the only marke to know this egg whether it be right or no, is this, that it will swim aloft above the water even against the stream, yea though it were bound and enchased with a plate of gold." But one must not be too severe upon Pliny, for he and his translator, Philemon Holland, provide an entertainment unequalled anywhere else.

To some extent the same applies to Plutarch of Chaeronea, who lived about the same time. Plutarch's writings, inspired as they were throughout by the desire to commend the ancient religion of Greece to a degenerate age, represent no milestone or turning-point in the history of embryology, yet there is a passage in the *Symposiaques, or Table-questions* which bears upon it. The third question of book 2 is "Whether was before, the hen or egg?" "This long time", says Plutarch, "I absteined from eating egges, by reason of a certaine dream I had, and the companie conceived an opinion or suspition of me that there were entred into my head the fantasies and superstitions of Orpheus or Pythagoras, and that I abhorred to eat an egge for that I believed it to be the principle and fountaine of generation." He then makes the various characters in the dialogue speak to the motion, and one of them, Firmus, ends his speech thus, "And now for that which remaineth (quoth he and therewith he laughed) I will sing unto those that be skilfull and of understanding one holy and sacred sentence taken out of the deepe secrets of Orpheus, which not onely importeth this much, that the egge was before the henne, but also attributeth and adjudgeth to it the right of eldership and priority of all things in the world, as for the rest, let them remaine unspoken of in silence (as Herodotus saith) for that they bee exceeding divine and mysticall, this onely will I speake by the way; that the world containing as it doth so many sorts and sundry kinds of living creatures, there is not in manner one, I dare well say, exempt from being engendred of an egge, for the egge bringeth forth birdes and foules that flie, fishes an infinit number that swimme, land creatures, as lizards, such as live both on land and water as crocodiles, those that bee two-footed, as the bird, such as are footlesse, as the serpent, and last of all, those that have many feet, as the unwinged locust. Not without great reason therefore is it consecrated to the sacred ceremonies and mysteries of Bacchus as representing that nature which produceth and comprehendeth in itselfe all things". This emphatic passage looks at first sight as if it was a statement of the Harveian doctrine *omne vivum ex ovo*. But the fact that no mammals are mentioned makes this improbable. Firmus then sits down and Senecius opposes him with the well-worn argument that the perfect must precede the imperfect, laying stress also on the occurrence of spontaneous, i.e. eggless, generation, and on the fact that men could find no "row" in eels. Three hundred years later, Ambrosius Macrobius handled the question again (see Whittaker), and the progress in embryological knowledge could be strikingly shown by the difference in treatment. It would be an interesting study to make a detailed comparison of them.

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[1] For further information about the serpent's eggs of the Druids, see Kendrick; they were probably fossil echinoderms.

1·6. Galen

Another fifty years brings us to Galen of Pergamos, second in greatness among ancient biologists, though in spite of his multitudinous writings he does not quite take this high rank in embryology. That knowledge of the development of the foetus was at this time specially associated with Peripatetic tradition appears from a remark of Lucian of Samosata, Galen's contemporary. In the satire called, *The Auction of the Philosophies*, Hermes, the auctioneer, referring to the Peripatetic who is being sold, says, "He will tell you all about the shaping of the embryo in the womb". But Galen was now to weld together all the biological knowledge of antiquity into his voluminous works, and so transmit it to the Middle Ages.

Most of Galen's writing was done between A.D. 150 and 180. Out of the twenty volumes of Kühn's edition of 1829, less than one is concerned with embryology, a proportion considerably less than in the case of Aristotle. Galen's embryology is to be found in his *περὶ φυσικῶν δυνάμεων*, *On the Natural Faculties*, which contains the theoretical part, and in his *On the Formation of the Foetus*, which contains the more anatomical part. There is also the probably spurious treatise *εἰ ζῷον τὸ κατὰ γαστρός*, *On the Question of whether the Embryo is an Animal.*

It is important to realise at the outset that Galen was a vitalist and a teleologist of the extremest kind. He regarded the living being as owing all its characteristics to an indwelling Physis or natural entity with whose "faculties" or powers it was the province of physiology to deal. The living organism according to him has a kind of artistic creative power, a *τέχνη*, which acts on the things around it by means of the faculties, *δυνάμεις*, by the aid of which each part attracts to itself what is useful and good for it, *τὸ οἰκεῖον*, and repels what is not, *τὸ ἀλλότριον*. These faculties, such as the "peptic faculty" in the stomach and the "sphygmic faculty" in the heart, are regarded by Galen as the causes of the specific functions or activity of the part in question. They are ultimate biological categories, for, although he admits the theoretical possibility of analysing them into simpler components, he never makes any attempt to do so, and evidently regards such an effort as doomed to failure, unlike Roux, whose "interim biological laws" are really conceived of as interim. "The effects of Nature", says Galen, "while the animal is still being formed in the womb are all the different parts of the body, and after it has been born an effect in which all parts share is the progress of each to its full size and thereafter the maintenance of itself as long as possible." Galen divides the effects of the faculties into three, Genesis, Growth, and Nutrition, and means by the first what we mean by embryogeny. "Genesis", he says, "is not a simple activity of Nature, but is compounded of *alteration* and of *shaping*. That is to say, in order that bone, nerve, veins, and all other tissues may come into existence, the underlying substance from which the animal springs must be altered; and in order that the substance so altered may acquire its appropriate shape and position, its cavities, outgrowths, and attachments, and so forth, it has to undergo a shaping or formative process. One would be justified in calling this substance which undergoes alteration the material of an animal, just as wood is the material of a ship and wax of an image." In this remarkable passage, Galen expresses modern views about chemical growth and chemical differentiation.

Galen then goes on to treat of embryogeny in more detail. "The seed having been cast into the womb or into the earth—for there is no difference—", he says (see p. 65), "then after a certain definite period a great number of parts become constituted in the substance which is being generated; these differ as regards moisture, dryness, coldness and warmth, and in all the other qualities which naturally derive therefrom", such as hardness, softness, viscosity, friability, lightness, heaviness, density, rarity, smoothness, roughness, thickness, and thinness. "Now Nature constructs bone, cartilage, nerve, membrane, ligament, vein, and so forth at the first stage of the animal's genesis, employing at this task a faculty which is, in general terms, generative and alterative, and, in more detail, warming, chilling, drying and moistening, or such as spring from the blending of these, for example, the bone-producing, nerve-producing, and cartilage-producing, faculties (since for the sake of clearness these terms must be used as well)....Now the peculiar flesh of the liver is of a certain kind as well, also that of the spleen, that of the kidneys and that of the lungs, and that of the heart, so also the proper substance of the brain, stomach, oesophagus, intestines and uterus is a sensible element, of similar parts all through, simple and uncompounded....Thus the special alterative faculties in each animal are of the same number as the elementary parts, and further, the activities must necessarily correspond each to one of the special parts, just as each part has its special use....As for the actual substance of the coats of the stomach, intestine, and uterus, each of these has been rendered what it is by a special alterative faculty of nature; while the bringing of these together, the combination therewith of the structures that are inserted into them, etc. have all been determined by a faculty which we call the shaping or formative faculty; this faculty we also state to be artistic—nay, the best and highest art—doing everything for some purpose, so that there is nothing ineffective or superfluous, or capable of being better disposed."

Thus the alterative faculty takes the primitive unformed raw material and changes it into the different forms represented by the different tissues, while the formative faculty, acting teleologically from within, organises these building-stones, as it were, into the various temples which make up the Acropolis of the completed animal. Galen next goes on to speak of the faculty of growth. "Let us first mention", he says, "that this too is present in the foetus *in utero* as is also the nutritive faculty, but that at that stage these two faculties are, as it were, handmaids to those already mentioned, and do not possess in themselves supreme authority."

Later on, until full stature is reached, growth is predominant, and finally nutrition assumes the hegemony.

So much for Galen's embryological theory. But before leaving the treatise *On the Natural Faculties*, it may be noted that he ascribes a retentive faculty to the uterus as well as to the stomach, and explains birth as being due to a cessation of action on the part of the retentive faculty, "when the object of the uterus has been fulfilled", and a coming into action of a hitherto quiescent propulsive faculty. This wholesale allotting of faculties can obviously be made to explain anything, and is eminently suited to a teleological account such as Galen's. It was not inconvenient as a framework within which all the biological knowledge of antiquity could be crystallised, but it was utterly pernicious to experimental science. Fifteen hundred years later it received what would have been the death-blow to any less virile theory, at the hands of Molière in his immortal *Malade Imaginaire*:

Bachelirius. Mihi a docto doctore
Demandatur causam et rationem quare
Opium facit dormire
A quoi respondeo
Quia est in eo
Virtus dormitiva
Cujus est nature
Sensus assoupire.
Chorus. Bene, bene, bene, bene respondere.
Dignus, dignus est entrare
In nostro docto corpore.
Bene, bene, respondere.

But to return to Galen. The book on the formation of the embryo opens with a historical account of the views of the Hippocratic writers with whom Galen was largely in agreement. It goes on to describe the anatomy of allantois, amnios, placenta, and membranes with considerable accuracy. The embryonic life consists, it says, of four stages: (1) an unformed seminal stage, (2) a stage in which the *tria principia* (a concept here met with for the first time) are engendered, the heart, liver and brain, (3) a stage when all the other parts are mapped out and (4) a stage when all the other parts have become clearly visible. Parallel with this development, the embryo also rises from possessing the life of a plant to that of an animal, and the umbilicus is made the root in the analogy with a plant. The embryo is formed, firstly, from menstrual blood, and secondly, from blood brought by the umbilical cord, and the way in which it turns into the embryo is made clearer as follows: "If you cut open the vein of an animal and let the blood flow out into moderately hot water; the formation of a coagulum very like the substance of the liver will be seen to take place". And in effect this viscus, according to Galen, is formed before the heart.

Galen also taught that the embryo excreted its urine into the allantois, and was acquainted with foetal atrophy. He gave a fairly correct account of the junction of the umbilical veins with the branches of the portal vein, and the umbilical with the iliac arteries, of the *foramen ovale*, the *ductus Arantii* and the *ductus Botalli*. He maintained that the embryo respired through the umbilical cord, and said that the blood passed in the embryo from the heart to the lungs and not *vice versa*. The belief that male foetuses were formed quicker than female ones he still entertained, and explained as being due to the superior heat and dryness of the male germ. He also associated the male conception with the right side and the female with the left and asserted that the intra-uterine movements are sooner felt in the case of the male than in the case of the female. Dry foods eaten by the mother, he thought, would lead to a more rapid development of the foetus than other kinds.

In this account of the Galenic embryology I have drawn not only upon the book on the formation of the foetus, but also upon his ὑπόμνημα, *Commentary on Hippocrates*, his περὶ αἰτίων συμπτωμάτων, *On the Causes of Symptoms*, and his book περὶ χρείας τῶν μορίων, *On the Use of Parts*. It is this latter work that had the greatest influence on the ages which followed Galen's life. In the course of seventeen books, he tries to demonstrate the value and teleological significance of every structure and function in the human and animal body, and to show that, being perfectly adapted to its end, it could not possibly be other in shape or nature than what it is. At the conclusion of this massive work with all its extraordinary ingenuity and labour, he says, "Such then and so great being the value of the argument now completed, this section makes it all plain and clear like a good epode—I say an epode, but not in the sense of one who uses enchantments (ἐπῳδαῖς) but as in the melic poets whom some call lyric, there is as well as strophe and antistrophe, an epode, which, so it is said, they used to sing standing before the altar as a hymn to the Gods. To this then I compare this final section and therefore I have called it by that name". This is one of the half-dozen most striking paragraphs in the history of biology; worthy to rank with the remarks of Hippocrates on the "Sacred Disease". Galen, as he wrote the words, must have thought of the altar of Dionysus in the Athenian or Pergamene theatre, made of marble and hung about with a garland, but they were equally applicable to the altar of a basilica of the Christian Church with the bishop and his priests celebrating the liturgy at it. What could be more charged with significance than this? At the end of the antique epoch the biology of all the schools, Croton, Akragas, Cos, Cnidus, Athens, Alexandria, Rome, is welded together and as it were deposited at the entrance into the sanctuary of Christendom. It was the turning-point, in Spengler's terminology, between Apollinian civilisation and Faustian culture. Galen's words are the more extraordinary, for he himself can hardly have foreseen that the long line of experimentalists which had arisen in the sixth century B.C. would come to an end with him. But so it was to be, and thenceforward experimental research and biological speculation were alike to cease, except for a few stray mutations, born out of due time, until in 1453 the city of Byzantium should burst like a ripe pod and, distributing her scholars all over the West, as if by a fertilising process, bring all the fruits of the Renaissance into being.

SECTION 2

EMBRYOLOGY FROM GALEN TO THE RENAISSANCE

2·1. Patristic, Talmudic, and Arabian Writers

We are now at the beginning of the second century A.D. The next thousand years can be passed over in as short a time as it has taken to describe the embryology of Galen alone. The Patristic writers, who on the whole were careful to base their psychology on the physiology of the ancients, had little to say about the developing embryo. Most of their interest in it was, as would naturally be expected, theological; Tertullian, for instance, held that the soul was present fully in the embryo throughout its intra-uterine life, thus denying that kind of psychological recapitulation which had been suggested by Aristotle. "Reply," he says in his *De Anima*, "O ye Mothers, and say whether you do not feel the movements of the child within you. How then can it have no soul?" These views were not held by other Fathers, of whom St Augustine of Hippo (*De Immortalitate et de quantitate animae*) may serve as a representative, for he thought that the embryo was "besouled" in the second month and "besexed" in the fourth. These various opinions were duly reflected in the law, and abortion, which had even been recommended theoretically by Plato and defended practically by Lysias in the fourth or fifth century B.C., now became equivalent to homicide and punishable by death. This fact leads Singer to the view that the Hippocratic oath is late, perhaps early Christian. The late Roman law, which, according to Spangenberg, regarded the foetus as not "*Homo*", not even "*Infans*", but only a "*Spes animantis*", was gradually replaced by a stern condemnation of all pre-natal infanticide. "And we pay no attention", said the Bishops of the Quinisext Council, held at Byzantium in 692, "to the subtle distinction as to whether the foetus is formed or unformed." Other authorities, following St Augustine, took a more liberal view, and the canon law as finally crystallised recognised first the fortieth day for males and the eightieth day for females as the moment of animation, but later the fortieth day for both sexes. The "*embryo informatus*" thus had no soul, the "*embryo formatus*" had, and as a corollary could be baptised. St Thomas Aquinas was of opinion that embryos dying *in utero* might possibly be saved: but Fulgentius denied it. As for the ancient belief that male embryos were formed twice as quickly as female ones, it lingered on until Goelicke took the trouble to disprove it experimentally in 1723.

Clement of Alexandria, in his book λόγος προτρεπτικὸς πρὸς Ἕλληνας, has some remarks to make on embryology, but adds nothing to the knowledge previously gained. He adopts the Peripatetic view that generation results from the combination of semen with menstrual blood, and he uses the Aristotelian illustration of rennet coagulating milk. Lactantius of Nicomedia, who lived about the date of the Nicene Council (A.D. 325) perpetuated the deeply-rooted association of male with right and female with left in his book *On the work of God, De opificio Dei.* He also maintained that the head was formed before the heart in embryogeny, and seems to have opened hen's eggs systematically at different stages, so that to this extent he was a better embryologist than Galen. St Gregory of Nyssa, as we have already seen (p. 20), evolved a neo-vitalistic theory which he applied to the growth of the embryo.

Late Latin writers, other than the theologians, do not say much about it. There is a passage in Ausonius, however, which describes the development of the foetus (*Eclog. de Rat. puerp.*) but it is almost wholly astrological. Elsewhere he says:

> juris idem tribus est, quod ter tribus; omnia in istis;
> forma hominis coepti, plenique exactio partu,
> quique novem novies fati tenet ultima finis.
> *Idyll* II (Gryphus ternarii numeri), 4–6.
>
> (The power of 3, in 3 times 3 lies too,
> Thus 9 rules human form and human birth,
> And 9 times 9 the end of human life.)

But this is probably a late echo of the Pythagoreans rather than an early prelude to Leonardo da Vinci and the mathematisation of nature.

That great mass of Jewish writings known as the Talmud, which grew up between the second and sixth centuries A.D., also contains some references to embryology, and certain Jewish physicians, such as Samuel-el-Yehudi, of the second century, are said to have devoted special attention to it. The embryo was called *peri habbeṭṭen* (fruit of the body), פרי הבטן. It grew through various definite stages:

(1) *golem* (formless, rolled-up thing), גולם, 0–1·5 months.
(2) *shefir meruqqām* (embroidered foetus), שפיר מרקם.
(3) ‘*ubbar* (something carried), עובר, 1·5–4 months.
(4) *walad* (child), ולד, 4–7 months.
(5) *walad shel qayāmā* (viable child), ולד של קיימא, 7–9 months.
(6) *ben she-kallu khadāshāw* (child whose months have been completed), בן שכלו חדשיו.

The ideas of the Talmudic writers on the life led by the embryo *in utero* are well represented by the remark, "It floateth like a nutshell on the waters and moveth hither and thither at every touch"

ואמר רבי אלעזר למה ולד דומה במעי אמו לאגוז מונח בספל של מים אדם נותן אצבעו עליו שוקע לכאן ולכאן.

And the classical passage, "Rabbi Simlai lectured: the babe in its mother's womb is like a rolled-up scroll, with folded arms lying closely pressed together, its elbows resting on its hips, its heels against its buttocks, its head between its knees. Its mouth is closed, its navel open. It eats its mother's food and sips its mother's drink: but it doth not excrete for fear of hurting"

דרש רבי שמלאי למה הולד דומה במעי אמו לפנקס שמקופל ומונח ידיו על שתי צדדיו שתי אציליו על ב׳ ארכבותיו וב׳ עקביו על ב׳ עגבותיו וראשו מונח לו בין ברכיו ופיו סתום וטבורו פתוח ואוכל ממה שאמו אוכלת ושותה ממה שאמו שותה ואינו מוציא רעי שמא יהרוג את אמו.

It was thought, moreover, that the bones and tendons, the nails, the marrow in the head and the white of the eye, were derived from the father, "who sows the white", but the skin, flesh, blood, hair, and the dark part of the eye from the mother, "who sows the red". This is evidently in direct descent from Aristotle through Galen, and may be compared with the following passage from the latter writer's *Commentary on Hippocrates*: "We teach that some parts of the body are formed from the semen and the flesh alone from blood. But because the amount of semen which is injected into the uterus is small, growth and increment must come for the most part from the blood". It might thus appear that, just as the Jews of Alexandria were reading Aristotle in the third century B.C., and incorporating

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him into the Wisdom Literature, so those of the third century A.D. were reading Galen and incorporating him into the Talmud. As for God, he contributed the life, the soul, the expression of the face, the functions of the different parts. This participation of three factors in generation, male, female, and god, is exceedingly ancient, as may be read in Robertson Smith. Some Talmudic writers held that development began with the head, agreeing with Lactantius, and others that it began at the navel, agreeing with Alcmaeon. Weber has given an account of the Talmudic beliefs about the infusion of the soul into the embryo. They do not seem to have embodied any new or striking idea.

Although the Talmud contained certain references of embryological interest, the first Hebrew treatise on biology was not composed till the tenth century, when Asaph Judaeus or Asaph-ha-Yehudi wrote on embryology about A.D. 950. His MSS. are exceedingly rare, but, according to Gottheil's description, they contain several sections on embryology. Steinschneider has given another description of them. For further details on the whole subject of Jewish embryology see Macht.

Arabian science, so justly famed for its successes in certain branches, was not of great help to embryology. Abū-l-Hasan 'Alī ibn Sahl ibn Rabbān al-Tabarī, a Moslem physician who flourished under the Caliphate of al-Mutawakkil about A.D. 850, wrote a book called *The Paradise of Wisdom*, in which an entire part was devoted to embryology, all the more interesting as it is a mixture of Greek and ancient Indian knowledge. Browne gives a description of it. Ibn Rabbān's contemporary, Thabit ibn Qurra, is also said to have written on embryology. The great Avicenna, or, to give him his proper name, Abū 'Alī-l-Hasan ibn 'Abdallāh ibn Sīnā, who lived from 978 to 1036, devoted certain chapters of his *Canon Medicinae* to the development of the foetus, but added nothing to Galen. His contemporaries, Abū-l-Qasim Maslama ibn Ahmad al-Majriti and Arib ibn Said al-Katib, a Spanish Moslem, wrote treatises on the generation of animals, but neither has survived.

What was alchemy doing all this time? It was engaged on many curious pursuits, but among them the interpretation of embryonic development was not one. Alchemical texts before the tenth century do make reference to eggs from time to time, but it is safe to say never with any trace of an interest in the development of the embryo out of them. One example taken from Berthelot's collection will suffice; it comes from the "6th book of the Philosopher" (Syriac).

> To make water of eggs
>
> Take as many eggs as you wish, break them and put the whites in a glass flask, place this in another vessel and surround it with fresh horse-dung up to the neck of the vessel. Leave it so for 15 days changing the dung every 5 days. Then distil the liquid in an alembic and taking a pound of the distillate add lime of eggs 2 ozs. Shake well and distil again. Do this 4 times. Take then of elixir of arsenic, 2 parts, of sulphur 1 part, of pyrites and magnesia, each 1 part. Pound in a mortar and add to the final distillate from the eggs. Do this for 7 days always working in the sunlight, once at sunrise, once in the middle of the day, and once at sunset. When this has been done, dry the mixture, pound it, and set it aside.

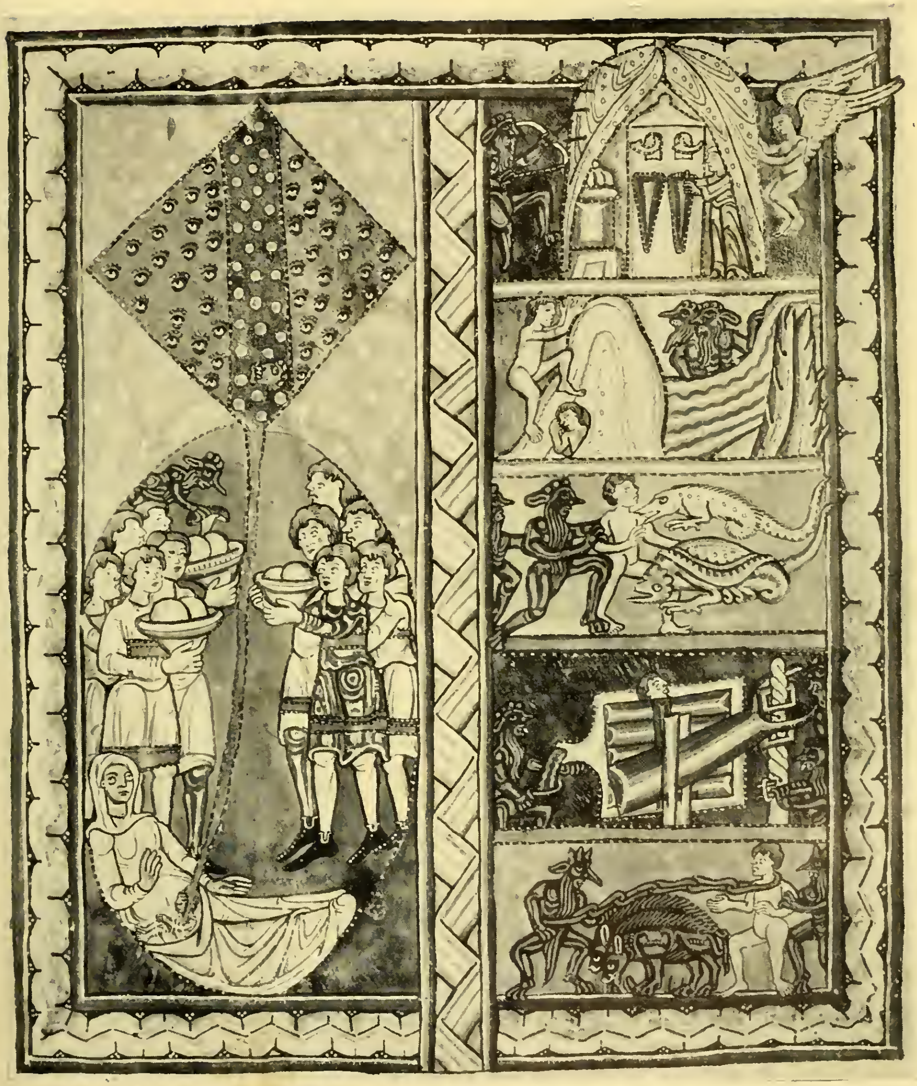
I could only find one reference to the embryo in a hen's egg among the vast number of alchemical directions of this time, and then only as a constituent of the egg which must be discarded. As we shall see, it is not until after the time of Paracelsus that the notion of applying chemical methods to eggs or embryos arises at all.

2·2. St Hildegard: the Lowest Depth

Not long after the death of Avicenna, St Hildegard was born. She lived from 1098 to 1180, and was Abbess successively of Disibodenberg and Bingen in the Rhineland. Her treatises on the world, which are an extraordinary medley of theological, mystical, scientific and philosophical speculation, have been described in detail by Singer, and, though in the books, *Liber Scivias* and *Liber Divinorum Operum simplicis hominis*, there is little of embryological interest, yet she does give an account of development and especially of the entry of the soul into the foetus.

This is illustrated in Plate III taken from the Wiesbaden Codex B of the *Liber Scivias*. The soul is here shown passing down from heaven into the body of the pregnant woman and so to the embryo within her. The divine wisdom is represented by a square object with its angles pointing to the four corners of the earth in symbol of stability. From it a long tube-like process descends into the mother's womb and down it the soul passes as a bright object, "spherical" or "shapeless", illuminating the whole body. The scene shows the mother in the foreground lying down; inside her there are traces of the foetal membranes; behind this ten persons are grouped, each carrying a vessel, into one of which a fiend pours some noxious substance from the left-hand corner. St Hildegard describes and expounds the scene as follows: "Behold, I saw upon earth men carrying milk in earthen vessels and making cheeses therefrom. Some was of the thick kind from which firm cheese is made, some of the thinner sort from which more porous cheese is made, and some was mixed with corruption and of the sort from which bitter cheese is made. And I saw the likeness of a woman having a complete human form within her womb. And then by a secret disposition of the most high craftsman, a fiery sphere having none of the lineaments of a human body possessed the heart of the form and reached the brain and transfused itself through all the members....And I saw that many circling eddies possessed the sphere and brought it earthward, but with ever renewed force it returned upwards and wailed aloud, asking, 'I, wanderer that I am, where am I?' 'In death's shadow.' 'And where go I?' 'In the way of sinners.' 'And what is my hope?' 'That of all wanderers.'... As for those whom thou hast seen carrying milk in earthen vessels, they are in the world, men and women alike, having in their bodies the seed of mankind from which are procreated the various kinds of human beings. Part is thickened because the seed in its strength is well and truly concocted and this produces forceful men to whom are allotted gifts both spiritual and carnal....And some had cheeses less firmly curdled, for in their feebleness they have seed imperfectly tempered and they raise offspring mostly stupid, feeble, and useless....And some was mixed with corruption...for the seed in that brew cannot be rightly raised, it is invalid, and makes misshapen men who are bitter distressed and oppressed of heart so that they may not lift their gaze to higher things....And often in forgetfulness of God and by the mocking devil a *mistio* is made of the man and the woman and the thing born therefrom is deformed, for parents who have sinned against me return to me crucified in their children".

We have already traced the wanderings of the cheese-analogy, which, beginning fresh with Aristotle, was taken to Alexandria and incorporated in the Wisdom Literature, and so found its way to the Arabic of 'Alī ibn a'l-Abbas al-Majūsī, or Haly-Abbas, as he was known in the West, a Persian. His *Liber Totius* appeared in Latin in 1523, but had been translated much earlier, at Monte Cassino between 1070 and 1085, by Constantine the African, who called it *Liber de Humana Natura*, and gave it out to be his own work. Thus



AN ILLUSTRATION FROM THE *LIBER SCIVIAS* OF ST HILDEGARD OF BINGEN (Wiesbaden Codex B) showing the descent of the soul into the embryo (*ca.* 1150 A.D.).

St Hildegard obtained it, and worked it up into one of her visions. At this point embryology touched, perhaps, its low-water mark. But a great man was at hand, destined to carry on the Aristotelian tradition and to add to it much of originality, in the shape of Albertus of Cologne. Before speaking of him, however, a word must be said about that very queer character, Michael Scot (1178–1234), who, according to Gunther, "appeared in Oxford in 1230 and experimented with the artificial incubation of eggs, having got an Egyptian to teach him how to incubate ostriches eggs by the heat of the Apulian sun". That "muddle-headed old magician", as Singer rightly calls him, was not the man to profit by it, but the point is interesting, especially as an Egyptian is mentioned. Haskins, in his curious studies of the scientific atmosphere of the court of the Emperor Frederick II of Sicily, has shown Scot, newly arrived from his alchemical studies in Spain, assisting that very learned and unorthodox monarch in his artificial incubation experiments.

2·3. Albertus Magnus

Albertus Magnus of Cologne and Bollstädt was born in 1206, and died in 1280, six years after his favourite disciple, St Thomas Aquinas. The greater part of his life was spent in study and teaching in one or other of the houses of the Dominican friars, to which he belonged, though for a time he was Bishop of Regensburg. Albert resembles Aristotle in many points, but principally because he produced biological work with, as it were, no antecedents. Just as Aristotle's contributions to embryology were preceded by no more than the diffuse speculations of the Ionian nature-philosophers, so Albert's came immediately after the dead period represented by the visions of St Hildegard. In many ways, Albert's position was much less conducive to good work than Aristotle's.

Albert follows Aristotle closely throughout his biological writings, quoting him word for word in large amounts, but the significant thing is that he does not follow him slavishly. He resembled Aristotle in paying much attention to the phenomena of generation, as a rough computation shows, Aristotle devoting 37 per cent. of his biological writings to this subject, and Albert 31 per cent., to which Galen's 7 per cent. may with interest be compared. Albert is extremely inferior to Aristotle, however, in point of arrangement; for Aristotle, although some of his books, such as the *De Generatione Animalium*, are sufficiently confused and repetitive, does yet succeed in infusing a clarity and incisiveness into his style. Albert, on the other hand, allows his argument to wander through his twenty-six books *De Animalibus* in the most complex convolutions, so that the sections on generation and embryology are found indiscriminately in the first, sixth, ninth, fifteenth, sixteenth, and seventeenth. In Book I he gives a kind of summary or skeleton of his views on the embryo. These follow Aristotle fairly closely; thus, he accepts the Aristotelian classification of animals according to their manner of generation, and thinks still that caterpillars are immature eggs; he derives the embryo from the white, not the yolk, and he explains why soft-shelled eggs, being imperfect, are of one colour only. But there are new observations; for instance, he describes an ovum *in ovo*, which he has seen, calling it a *natura peccatis*, and he speaks definitely of the seed of the woman, thus departing from Peripatetic opinion, and adopting the Epicurean view. The female seed, he thinks, suffers coagulation like cheese by the male seed, and to these two humidities there must be added a third, namely, the menstrual blood (corresponding to the yolk in the case of the bird). "When these three humidities therefore have been brought into one place, all the similar members except the blood and fat are formed from the two humidities of which one generates actively but the other passively. But the blood which is attracted for the nutriment of the embryo is double in virtue and double in substance. For a certain part of the blood is united with the sperm in such a way that it takes on some of the virtue of the seed because a certain part of the spermatic humour remains in it and from this are begotten the teeth and for this reason they grow again if they are pulled out at an age near the time of sperm-making and do not grow again at an age remoter from this, at which the virtue of the first generating principle has vanished from the blood. But another part of the blood is of twofold or threefold substance and from the thick part of the blood itself is generated the flesh. And this flows in and flows out and grows again if rubbed away. From the watery part of the same blood or of the nutritive humour are generated the fat and oil and this flows in and out more easily than the flesh itself, but other parts of the blood are its refuse and impurities and are not attracted to the generation of any part of the animal, but having been collected until birth are expelled with the embryo from the uterus in the foetal membranes, like the remnants in the hen's egg after the chick has hatched. There is a similar virtue in the liver and heart of animals which organs after the animals are born form the flesh and fat from food in accordance with its twofold substance, and expel the refuse as we said before."

In the sixth book, Albert contradicts Aristotle's opinion that male chick develops out of the sharp-ended egg, and one hopes that he is going to say there is no relationship between egg-shape and sex, but no, he goes on to say that the Aristotelian statement rested on a textual error (in which he was quite wrong), so that really Aristotle agreed with Avicenna in saying that the males always develop from the more spherical eggs because the sphere is the most perfect of figures in solid geometry. These errors had a most persistent life: Horace has a passage in which they appear—

longa quibus facies ovis erit, illa memento
ut suci melioris, et ut magis alma rotundis
ponere: namque marem cohibent callosa vitellum.

(When you would feast upon eggs, make choice of the long ones; they are whiter and sweeter and more nourishing than the round, for being hard they contain the yolk of the male.)

They were finally abolished by two naturalists, Günther and Bühle, who took the trouble to disprove them experimentally in the eighteenth century. Albertus refers here to artificial incubation: "For the alterative and maturative heat", he says, "of the egg is in the egg itself and the warmth which the bird provides is altogether external [*extrinsecus est amminiculans*] since in certain hot countries the eggs of fowls are put under the surface of the earth and come to completion of their own accord, as in Egypt, for the Egyptians hatch them out by placing them under dung in the sunlight". Next he speaks of monsters and of the modes of corruption of eggs which he divides into four: (1) decomposition of white, (2) decomposition of yolk, (3) bursting of the yolk-membrane, (4) *antiquitas ovi*. "And from the second cause it sometimes happens", he says, "that in the corruption of the humours certain igneous parts are carried blazing to the shell of the egg and distribute themselves over it so that it shines in the dark like rotten wood; as happened in the case of that egg[1] which Avicenna said he saw in the city called Kanetrizine in the country of the Corascenes." Albert is inclined to think that astrological influences may have an effect on foetal life, but he treats the suggestion with considerable scepticism, although he believes that thunder and lightning kill the embryos of fowls (a popular belief to which Féré tried not long ago to give a scientific foundation), and he regards the embryo of the crow as especially susceptible, though on what grounds he does not say.

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[1] See on this subject Zäch.

The fourth chapter of the first tractate of the sixth book contains Albert's description of development of the chick, and is extremely interesting. He makes two principal mistakes: (*a*) he describes a quite non-existent fissure in the shell by which the chick may emerge, (*b*) he maintains that the yolk ascends after a day or two into the sharp end of the egg, adducing as the reason that there is found there more heat and formative force than elsewhere. On the other hand, he correctly describes (*a*) the pulsating drop of blood on the third day, and (*b*) he identifies it with the heart with its *systolen et dyastolen* sending out the "formative virtue" to all the parts of the growing body. He notices (*c*) that the differentiation of the chick at first proceeds rapidly and later more slowly. But the most notable characteristic of Albert's embryology is the way in which he is hampered by his inability to invent a technical terminology. Singer has studied the way in which anatomical terms, such as "syrach", etc., came into use, but whatever the causes were which produced them, they did not operate much in Albert's mind. He represents the point beyond which embryology could not advance, until it had created a new set of terms. This is well illustrated by the following passage:

> "But from the drop of blood", he says, "out of which the heart is formed, there proceed two vein-like and pulsatile passages and there is in them a purer blood which forms the chief organs such as the liver and lungs and these though very small at first grow and extend at last to the outer membranes which hold the whole material of the egg together. There they ramify in many divisions, but the greater of them appears on the membrane which holds the white of the egg within it [the allantois]. The albumen, at first quite white, is changed owing to the power of the vein almost to a pale yellow-green tint [*palearem colorem*]. Then the path of which we spoke proceeds to a place in which the head of the embryo is found carrying thither the virtue and purer material from which are formed the head and the brain, which is the marrow of the head. In the formation of the head also are found the eyes and because they are of an aqueous humidity which is with difficulty used up by the first heat they are very large, swelling out and bulging from the chick's head. A short

time afterwards, however, they settle down a little and lose their swelling owing to the digestive action of the heat—and all this is brought about by the action of the formative virtue carried along the passage which is directed to the head, but before arriving there is separated and ramified by the great vein of the albumen-membrane, as may be clearly seen by anyone who breaks an egg at this time and notes the head appearing in the wet part of the egg and at the top of the other members. For what appears first in the making of a foetus are the upper parts because they are nobler and more spiritual being compacted of the subtler part of the egg wherein the formative virtue is stronger. When this happened one of the aforementioned two passages which spring from the heart branches into two, one of them going to the spiritual part which contains the heart and divides there in it carrying to it the pulse and subtle blood from which the lungs and other spiritual parts are formed, and the other going through the diaphragm [*dyafracma*] to enclose within it at the other end the yolk of the egg, around which it forms the liver and stomach. It is accordingly said to take the place of the umbilicus in other animals and through it food is drawn in to supply the flesh for the chick's body, for the principle of generation of the radical members of the chick comes from the albumen but the food from which is made the flesh filling up all the hollows is from the yolk."

After ten days, Albert goes on to say, all the constituent organs are mapped out and the head is greater then than the rest of the body put together. He observes that the yolk liquefies early in development and that slimy concretions are present in the allantoic fluid later on (uric acid). But the passage quoted does demonstrate that before further progress could be made some better name must be found than "the interior membrane to which the first vessel proceeds" for a given structure.

Albert, however, was accomplishing a good work. One of his best amplifications of Aristotle was his description of the relationship between yolk and embryo in fishes. Just as his words about the chick demonstrate that he must have opened hen's eggs at different stages during incubation, so his words about fish eggs show that he must have dissected and examined them also. Thus (Book VI, tractate 2, chap. i) he says, "Between the mode of development [*anathomiam generationis*] of birds' and fishes eggs there is this difference; during the development of the fish the second of the two veins which extend from the heart does not exist. For we do not find the vein which extends to the outer covering of the eggs of birds which some wrongly call the umbilicus because it carries the blood to the outside parts, but we do find the vein which corresponds to the yolk vein of birds, for this vein imbibes the nourishment by which the limbs increase.

Therefore the generation of the fish embryo begins from the sharp end of the egg like that of birds and channels extend from the heart to the head and eyes and first in them appear the upper parts. As the growth of the young fish proceeds the yolk decreases in amount being incorporated into the members and it disappears entirely when development is complete. The beating of the heart, which some call panting, is transmitted through the pulsating veins to the lower part of the belly carrying life to the inferior members. While the young fish are small and not yet fully developed they have veins of great length which take the place of the umbilicus, but as they grow these shorten till they contract into the body by the heart as has been said about birds. The young fish are enclosed in a covering just like the embryos of birds, which resembles the *dura mater* and beneath it another containing the foetus and nothing else, while between the two there is the moisture rejected during the creation of the embryo". Albert also described ovoviviparous fishes but it is more difficult in that case to tell whether he had himself seen and dissected them. He notes also the prodigality of nature in producing so many marine eggs only destined to be eaten.

In Books IX and XV he treats of the Galenic views on generation and insists again that there is a seed provided by the female. In Book XVI he gives his opinions about the animation of the embryo, quoting the views of the ancients as given in Plutarch, e.g. Alexander the Peripatetic, Empedocles, Anaxagoras, Theodorus and Theophrastus, the Peripatetics, Socrates, Plato, the Stoics, Avicenna, and Aristotle, "who saw the truth", but—and it is interesting to notice it—never the Christian Fathers, whose writings must have been well known to him. In discussing the Aristotelian views he compares the menstrual blood to the marble and the semen to the man with a chisel in his hand.

On the question of epigenesis and preformation, he follows Aristotle almost word for word, using the same analogies, such as the "dead eye" and the sleeping mathematician. Here his scholasticism comes out clearly, for in rejecting altogether the theory that one part being formed then forms the next part, he says, not that *A* would have to be in some way like *B*, but is not, as Aristotle had, but simply "*Generans et generatum, est simul esset et non esset, quod omnino est impossibile*"—a high-handed and very unscientific manner of settling the question. In conformity with his theology and in contradistinction from Aristotle he makes the vegetative and sensitive souls arrive automatically into the embryo but the rational soul only by a direct act of God.

His mammalian embryology presents some points of interest. He follows Hippocrates ("Ypocras") in an account of the co-operation of heat and cold in member-formation, and he holds very enlightened views about foetal nutrition. "It appears therefore that the embryo hangs from the cord and that the cord is joined with the vein and that the vein extends through the uterus and has blood running through it to the foetus like water through a canal. Round the embryo there are membranes and webs as we have seen. But those who think that the embryo is fed by little bits of flesh through the cord are wrong and lie, because if this were the case with man it would happen also with other animals and that it does not do so anybody can find out by investigation [*per anathomyam*]."

Finally, it is typical that in Book XVII Albert repeats what he has already said in Book VI about the generation of the hen out of the egg all over again with slight changes, but he adds the significant biochemical remark that "eggs grow into embryos because their wetness is like the wetness of yeast". The importance of Albert in the history of embryology is clear. With him the new spirit of investigation leapt up into being, and, though there were many years yet to pass before Harvey, the modern as opposed to the ancient period of embryology had begun. Albert's writings were often copied and printed in the next few centuries, and even as late as 1601 *De Secretis Mulierum*, an epitome of his books on generation, was published. In some sense, it still is, as it forms the backbone of the little book *Aristotle's Masterpiece*, of which thousands of copies are sold in England every year. The copy of the *De Secretis* in the Caius College Library has written across the title-page in faded ink "*Simulacra sanctitas, duplex iniquitas, Nathan Emgross, Nov.* 20. 1613." But in spite of Mr Emgross, Albertus, rightly called Magnus, has had the happy fate of being beatified both by the Church and by science.

2·4. The Scholastic Period

St Thomas Aquinas (1227–1274) incorporated the Aristotelian theories of embryology into his *Summa Theologica* especially under the head *De propagatione hominis quantum ad corpus*. There are some striking passages, such as "The generative power of the female is imperfect compared to that of the male; for just as in the crafts, the inferior workman prepares the material and the more skilled operator shapes it, so likewise the female generative virtue provides the substance but the active male virtue makes it into the finished product". How admirably this expresses the dominating sentiment of the Middle Ages! Aristotle might make a distinction between matter and form in generation, but the mediaeval mind, with its perpetual hankering after value, would at once enquire which of the two was the higher, the nobler, the more honourable.

St Thomas' theory of embryonic animation was complicated. He had a notion that the foetus was first endowed with a vegetative soul, which in due course perished, at which moment the embryo came into the possession of a sensitive soul, which died in its turn, only to be replaced by a rational soul provided directly by God. This led him into great difficulties, for, if this scheme were true, it was difficult to say that man generated man at all; on the contrary he could hardly be said to generate more than a sensitive soul which died before birth, and, on this view, what was to happen to original sin? As Harris has put it, Plato had said that the intellect was the man, using the body as a boatman uses a boat. Averroes had said precisely the opposite, namely, that the essence of humanity was in the body, and that the intellect was something extrinsic, not limited to the individual, but common to the race. Aristotle had taken the middle position, and given a soul to plants and animals, but, in doing so, he had made it into a vital rather than a psychological principle. The task of combining this ψυχή with the *anima* of the Fathers was what scholastic philosophy had before it. No wonder that St Thomas' account of embryonic animation was open to criticism. An echo of it appears in a poem of Jalalu'd-Din Rumi (1207–1273), the greatest of the Persian Sufi poets, and an exact contemporary of St Thomas Aquinas:

> I died from mineral and plant became:
> Died from the plant, and took a sentient frame;
> Died from the beast, and donned a human dress;
> When by my dying did I e'er grow less?

Duns Scotus (1266–1308) objected to St Thomas' theory on the grounds already mentioned, and he himself abandoned the vegetative and sensitive souls altogether in his *De Rerum Principio*. This solution was no better than that of St Thomas, for, agreeing with the latter as Duns did that the rational soul was not an ordinary form "educed" from the "potentiality" of the material, but rather an *ad hoc* creation of God, injected by divine power into the embryo at the appropriate moment, it was difficult to see how the spiritual effects of Adam's fall could be transmitted to the men of each generation. It was as if only acquired characteristics were inherited. But the further course of theological embryology need not be pursued here; it runs in every century parallel with true scientific embryology, and it is not my purpose to do more than take a glance at its progress from time to time.

In the *Speculum Naturale*, which was written about 1250, by Vincent of Beauvais, the embryology of Constantine the African appears again, and the embryology of Aristotle, Galen, and the scholastics is to be found in Dante Alighieri (1265–1321), who dealt with the subject in his *Convivio*, and especially in the *Divina Commedia*. In Canto xxv of the *Purgatorio*, Statius (the personification of human philosophy enlightened by divine revelation) is made to speak to the poet thus: "If thy mind, my son, gives due heed to my words and takes them home, they will elucidate the question thou dost ask. Perfect blood which is in no case drawn from the thirsty veins, but which remains behind like food that is removed from table, receives in the heart informing power for all the members of the human body, like the other blood which courses through the veins in order to be converted into those members. After being digested a second time it descends to the part whereof it is more seemly to keep silence than to speak, and thence it afterwards drops into the natural receptacle (the uterus) upon another's blood; there the one blood and the other mingle. One is appointed to be passive, the other to be active according to the perfect place whence it proceeds (the heart). And being united with it, it begins to operate, first by coagulating it, and then by vivifying that to which it has given consistency, so that there may be material for it to work upon [*e poi avviva, Ciò che per sua materia fe' constare*]. The active power having become a (vegetative) soul like that of a plant—only differing from it in this, that the former is in progress while the latter has reached its goal—thereafter works so much that it moves and feels like a sea-fungus and as the next stage it takes in hand to provide with organs the faculties which spring from it. At this point, my son, the power which proceeds from the heart of the begetter is expanded and developed, that power in which

Nature is intent on forming all the members, but how from being an animal it becomes a child, thou seest not yet, moreover this is so difficult a point that formerly it led astray one more wise than thou [Averroes], so that in his teaching he separated the active 'intellect' from the soul because he could not see any organ definitely appropriated by it. Open thy heart to the truth and know that as soon as the brain of the foetus is perfectly organised, the Prime Mover, rejoicing in this display of skill on the part of Nature, turns him towards it and infuses a new spirit replete with power into it which subsumes into its own essence the active elements which it finds already there, and so forms one single soul which lives and feels and is conscious of its own existence. And that thou mayst find my saying less strange, bethink thee how the heat of the sun passing into the juice which the grape distils, makes wine".

Having said this, Statius, Virgil and Dante pass on to the seventh ledge in Purgatory. It is interesting to see how Dante emphasises the dynamic teleological side of Aristotle and practically speaks of the soul enfleshing itself and arranging organs for its faculties. The reference to Averroes is explained by the fact that Averroes was a Traducianist, and held that all the soul was generated by man at the same time as the body, whereas both St Thomas and Dante, as Creationists, held that each fresh soul was a special creation of God inserted by him into the brain of the embryo. The mention of Dante's contemporary, Mondino de Luzzi (1270–1326), brings us to the more practical aspects of embryology at this period. Mondino is the most outstanding figure among the Bolognese anatomists of what is really the first period of the revival of biology. After him, as we shall see, biology languished for a couple of centuries until the advent of such men as Ulysses Aldrovandus in the sixteenth century, and Singer has shown that this was probably due to the fact that anatomy professors did not dissect in person. *A fortiori* embryotomy was infrequent.

But Mondino's *Anathomia*, published in 1316, contained statements about the organs of generation which were rather important. He retains the notion of the seven-celled uterus, which had been introduced by Michael Scot, but he adopts a reasonable compromise between the opinions of Galen and Aristotle on the physiology of embryo formation. The distance between him and Leonardo da Vinci (1452–1519) would, however, be estimated rather at five or six centuries than at the century and a quarter that it actually was.

2·5. Leonardo da Vinci

Leonardo was not alone among the artists of the Renaissance in his anatomical interests, for Michael Angelo, Raphael, Dürer, Mantegna, and Verrochio all made dissections in order to increase their knowledge of the human body. But he penetrated more curiously into biology than they did, and he will always remain one of the greatest of biologists, for he first introduced the quantitative outlook. In this he was some four hundred years before his time.

Leonardo's embryology is contained in the third volume of his notebooks, *Quaderni d' Anatomia*, published in facsimile by the admirable labours of three Norwegian scholars, Vangensten, Fohnahn and Hopstock, in 1911. His notebooks are a remarkable, and, indeed, charming miscellany of anatomical drawings, physiological diagrams, architectural and mechanical sketches and notes such as "Shirts, hose, and shoes", "Go and see Messer Andreas", "get coal", "the supreme fool (is the) necromancer, and enchanter".

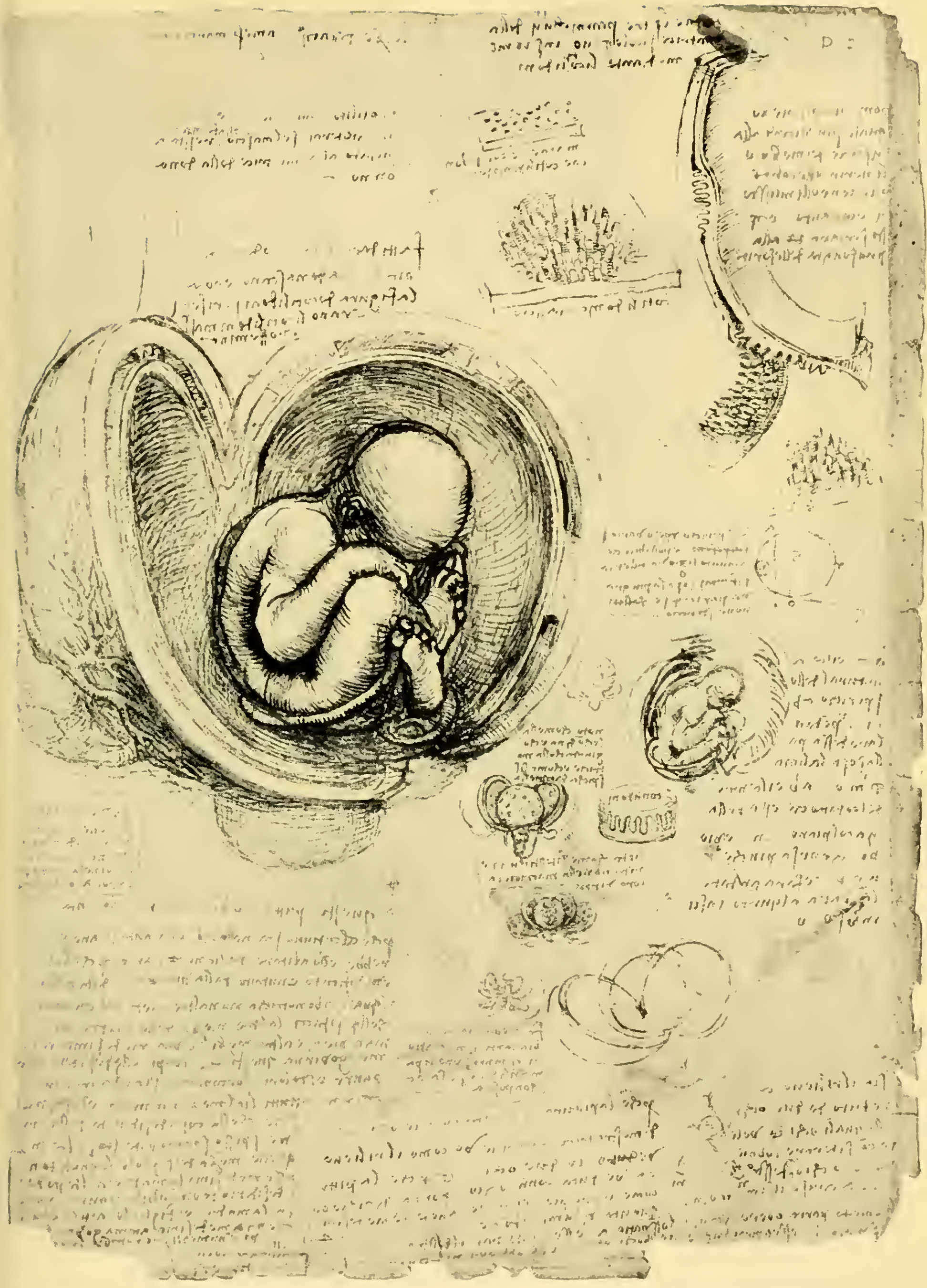
His dissections of the pregnant uterus and its membranes are beautifully depicted, as can be seen from the figures which are here reproduced (Plate IV). He was acquainted with amnios and chorion, and he knew that the umbilical cord was composed only of vessels, though he seems to have thought the human placenta was cotyledonous. There is one drawing which the editors suppose to represent the developing hen's egg, but I do not feel that this ascription is likely. Indeed, Leonardo worked with eggs much less than with mammalian embryos, though there are references to the former. "See how birds are nourished in their eggs", he says in one place, to remind himself, perhaps, of possible experiments, and, elsewhere, "Chickens are hatched by means of the ovens of the fireplace". Again, "Ask the wife of Biagino Crivelli (was she the Lucrezia Crivelli, whose portrait Leonardo painted?) how the capon rears and hatches the eggs of the hen when he is inebriated", a subject recently reopened by Lienhart. "You must first dissect the hatched egg before you show the difference between the human liver in foetus and adult." Leonardo perpetuates a persistent error in the note, "Eggs which have a round form produce males, those which have a long form produce females".

Concerning the mammalian foetus, he says, "The veins of the child do not ramify in the substance of the uterus of its mother but in the placenta which takes the place of a shirt in the interior of the uterus which it coats and to which it is connected but not united by means of the cotyledons". Thus in one sentence Leonardo falls into a mistake in saying that the human placenta is cotyledonous, but at the same time asserts a fact which it took all the ingenuity of the seventeenth century to prove to be true, namely, that the foetal circulation is not continuous with that of the mother, for the placenta is only connected to the uterine wall and not united with it. "The child", Leonardo goes on to say, "lies in the uterus surrounded with water, because heavy things weigh less in water than in the air and the less so the more viscous and greasy the water is. And then such water distributes its own weight with the weight of the creature over the whole body and sides of the uterus." The tendency towards quantitative and mathematical explanations is apparent at once.

Further notes are, "Note how the foetus breathes and how it is nourished through the umbilical cord and why one soul governs two bodies, as you see the mother desiring food and the child remaining marked (by a given amount of growth) because of it. Avicenna pretends that the soul generates the soul and the body the body. *Per errata*". The child, says Leonardo, secretes urine while still *in utero*, and has excrement in its intestines; at four months it has chyle in its stomach, made perhaps from menstrual blood. But it has no voice *in utero*, "when women say that the foetus is heard to weep sometimes within the uterus, this is rather the sound of some flatus . . .". Nor does it breathe there (on this point Leonardo contradicts himself). "The child does not respire within the body of its mother because it lies in water and he who breathes in water is immediately drowned." "Breathing is not necessary to the embryo because it is vivified and nourished by the life and food of the mother." Nor does the embryonic heart beat. To us the statement that there is no respiration in the uterus is obviously false, but we mean by the word tissue respiration, whereas in Leonardo's time pulmonary respiration was intended; he was therefore perfectly right in denying that the embryo breathed, as certain anatomists before him had asserted.

His only reference to the soul runs thus: "Nature places in the bodies of animals the soul, the composer of the body, i.e. the soul of the mother, which first composes, in the womb, the shape of man and in due time awakens the soul which shall be the inhabitant thereof, which first remains asleep and under the tutelage of the soul of the mother which through the umbilical vein nourishes and vivifies

PLATE IV



A PAGE FROM LEONARDO DA VINCI'S ANATOMICAL NOTEBOOKS
(*QUADERNI D' ANATOMIA*), *ca.* 1490 A.D.

it". This is not very revolutionary. But Leonardo was the first embryologist to make any quantitative observations on embryonic growth; he defined, for instance, the length of a full-grown embryo as one braccio and the adult as three times that. "The child", he says, "grows daily far more when in the body of its mother than when it is outside of the body and this teaches us why in the first year when it finds itself outside the body of the mother, or, rather, in the first 9 months, it does not double the size of the 9 months when it found itself within the mother's body. Nor in 18 months has it doubled the size it was 9 months after it was born, and thus in every 9 months diminishing the quantity of such increase till it has come to its greatest height." Here Leonardo touches on one of the most modern quantitative aspects of embryology, and one almost expects to see him exemplify his words with a graph until one remembers with a shock that he lived two centuries before Descartes and five before Minot. His numerical data may also have included figures about the relative sizes of the parts, and the germ of the line of research so successfully pursued by Scammon in our own times may be found in the note "The liver is relatively much larger in the foetus than in the grown man". Other quantitative notes concern the length of the embryonic intestines as in the laconic "20 braccia of bowels" and the statement that "the length of the umbilical cord always equals the length of the foetal body in man though not in animals".[1]

He said little about heredity, but in one place he mentions a case of sexual intercourse between an Italian woman and an Ethiopian, the outcome of which assured him that blackness was not due to the direct action of the sun and that the "seed of the female was as potent as that of the male in generation". Finally, the best instance of the wideness of his thought appears in the note, "All seeds have an umbilical cord which breaks when the seed is mature. And similarly they have matrix and secundines as the herbs and all the seeds which grow in shells show". We have met this idea before in Hippocrates of Cos, and we shall find it again in Nathaniel Highmore.

It is no coincidence that pictures of weights and cogs and pulleys stand side by side in Leonardo's notes with anatomical drawings of the embryo. As Hopstock says, "Leonardo arrives at the conclusion that there is but one natural law which governs the world, Necessity.

[1] Leonardo would have enjoyed Fog's statistical study of 8000 umbilical cords (1930).

Necessity is Nature's master and guardian, it is Necessity that makes the eternal laws". If Aristotle is the father of embryology regarded as a branch of natural history, Leonardo is the father of embryology regarded as an exact science.

2·6. The Sixteenth Century: the Macro-Iconographers

After such a man, the writings of his contemporaries, such as the mythical Johannes de Ketham, Alessandro Achillini and Gabriele de Gerbi, appear beyond description inferior. De Ketham's embryology has been described by Ferckel. De Gerbi included in his *Liber Anatomiae corporis humani et singulorum membrorum illius* a section entitled *De Generatione Embryonis*, but there is nothing to be said about it except that it is a verbose compilation of the views of Aristotle and Galen taken from Avicenna. The work of Nolanus in 1532 presents certain points of interest, but it is of little importance. Petrus Crescentius in his work on husbandry of 1548 mentions artificial incubation in ovens, but rather as a lost art. About this time also Hieronymus Dandinus Cesenas, a Jesuit, wrote a treatise on Galen's division of organs into white and red, those proceeding from the semen and those proceeding from the blood: it is cited by Aldrovandus, but I have not been able to consult it.

The most remarkable feature of the first half of the century was the encyclopaedic group of zoologists which now arose. Thus Belon and Rondelet, whose well-illustrated catalogues of animals were appearing from 1550 onwards, did a good service to comparative embryology in figuring the ovoviviparous selachians and viviparous cetacea. Gesner belongs to this group. All of them reproduce thin versions of Aristotle, when they speak of generation as such, and this is what differentiates them from Ulysses Aldrovandus, of whom I shall speak presently. Figs. 3 and 4 show Rondelet's pictures of a viviparous dolphin and an ovoviviparous selachian.

But the end of the twilight period was now at hand, for, within thirty years after the death of de Gerbi in 1505, four great embryologists were born as well as the greatest anatomist of any age, Andreas Vesalius (1514), of whom I shall say no more, for he had no opportunities for dissecting human embryos, and took hardly any interest in foetal development. But in 1522 Ulysses Aldrovandus was born, and in the following year Gabriel Fallopius, in 1530 Julius Caesar Arantius and in 1534 Volcher Coiter. Only three more years bring us to the birth of Andreas Laurentius and of Hieronymus Fabricius ab Aquapendente, the teacher of William Harvey.

The senior member of this group, Ulysses Aldrovandus, was the first biologist since Aristotle to open the eggs of hens regularly during their incubation period, and to describe in detail the appearances which he found there. In his *Ornithologia*, published at Bonn in 1597, he set out to describe all the known kinds of birds, discussing in turn not only their zoological and physiological characteristics, but also their significance as presages and for augury, their mystical meaning, their use as allegories and for eating, and finally all the legends respecting them. *Generositas*, *Temperantia*, *Liberalitas*, *aquilae* one finds. Beginning with the eagle, he proceeds to the vulture, the owl, the bat (the only viviparous bird!), the ostrich, the harpy(!),

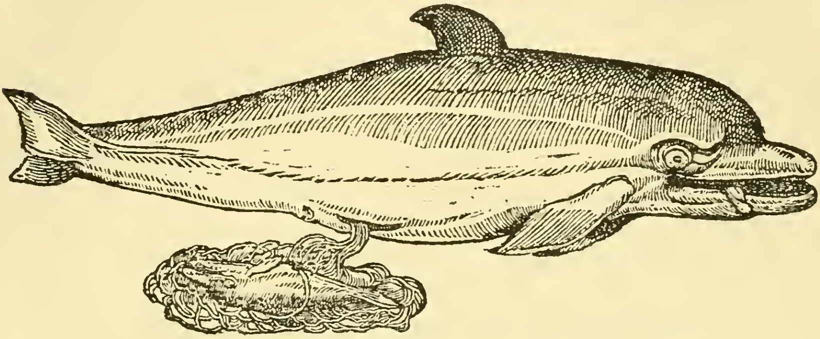


Fig. 3. A (viviparous) dolphin: from Rondelet's *De piscibus marinis* of 1554.

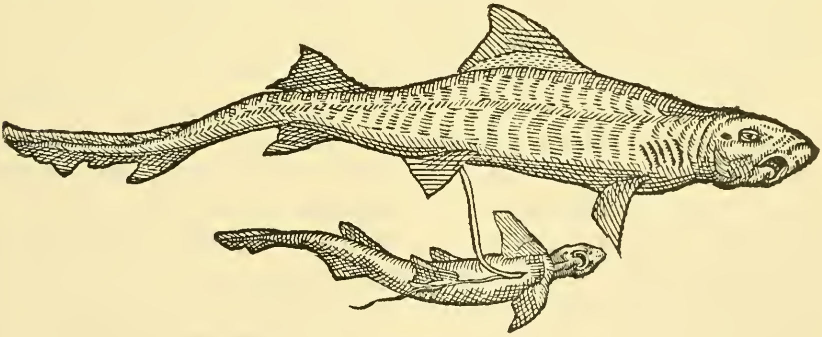


Fig. 4. An (ovoviviparous) shark: from Rondelet's *De piscibus marinis* of 1554.

the parrot, the crow, and so to the fowl. Side by side with a reference to the famous poem of Prudentius (*Multi sunt Presbyteri*, translated by J. M. Neale) about the steeple-cock, we find an excellent account of the generation of the chick in the egg. The book is illustrated sumptuously, but unfortunately there is only one picture of embryological interest, namely, a chick in the act of hatching.

In Aldrovandus' embryology there is much discussion of Aristotle and Galen, but traces of an independent spirit abound. Pliny's view that the heart was formed in the white is "exploded", and Aldrovandus says that it is formed on the yolk-membrane. He refutes the opinion of Galen also that the liver is first formed, in connection with which he says, "In order that I might bring to an end this controversy between the philosophers and the physicians I followed with the keenest curiosity and diligence the incubation of 22 hen's eggs, opening one each day; thus I found Aristotle's doctrine to be the truest. And because apart from the fact that these matters are most worthy of being looked into they provide also the greatest pleasure and entertainment I have thought it well to describe them as clearly and briefly as possible".

Aldrovandus also contradicts Albertus, and propounds a new theory, namely, that the *spiritualia* (the organs in the thorax) are formed from the seed of the cock (*ex maris semine sunt*). This seed he affirms to be present in the egg, and he identifies it with the chalazae, thus anticipating Fabricius ab Aquapendente, but not going quite so far, and explicitly opposing Gaza, who had said not long before that the chalazae were simply congealed water. Aldrovandus' admiration for Aristotle is extreme, and, though he differs from him about the chalazae, he defends the Aristotelian opinion that the chick was made from the white but nourished from the yolk. His argument for this is new, however; it is that, during incubation, the latter liquefies but the former hardens; now in all digestion liquefaction takes place, and in all growth hardening, therefore, etc. This argument is a great deal more cogent than most of those which were current between 1550 and 1650. He goes out of his way to castigate Albertus for saying that the yolk moves up into the sharp point of the egg, for experience assures him that it does not, "as I have observed by cutting open an egg after one day's incubation". A striking instance of his powers of observation was his description of the "egg-tooth" of embryonic birds, a discovery made anew in the nineteenth century by Yarrell and Rose. The chick was perfect in form, according to him, on the tenth day.

The peculiarity of Aldrovandus lies in the fact that he incorporated so many elements into one book, and was able to produce a collection of chapters in which good scientific observation sat at the closest quarters with literary allusion and semi-theological homily. So well-proportioned a mixture as the *Ornithologia* is not often found. As a final instance three consecutive paragraphs may be mentioned, in the first of which he discusses Plutarch's arid problem about the priority of egg or hen, next he makes some very reasonable remarks about teratology, suggesting that monsters come from yolks which are physico-chemically abnormal in some way, while in the third he expresses strong scepticism concerning the tale that the basilisk is sometimes hatched out from a hen's egg—"*Ego ne jurantibus quidem crediderim*", he says. This last notion is found in the fourteenth-century poem of Prudentius alluded to above, and appears again in the *Miscellaneous Exercitations* of Caspar Bartholinus the younger, whose second chapter is devoted to showing "That the basilisk hatcheth not from the egg of the hen", a conclusion which has been amply confirmed in the light of subsequent experience. Bartholinus gives a bibliography of this curious legend.

Aldrovandus and his disciple Volcher Coiter the Frisian, as he described himself, were alike in not suffering from the prevailing vice of the age, verbosity. Coiter's *Externarum et Internarum principalium humani corporis partium tabulae et exercitationes*, which appeared at Nuremberg in 1573—a beautifully printed book—contained a brief section entitled *De ovorum gallinaceorum generationis primo exordio progressuque et pulli gallinacei creationis ordine.* His Latin style betrays his German origin, for the constructions are very Teutonic, although the meaning is always perfectly clear. Coiter says, "In the year 1564 in the month of May at Bologna, being instigated by that excellent professor of philosophy outstanding in varied sciences and arts, Doctor Ulysses Aldrovandus, and by other doctors and students, I ordered 2 broody fowls to be brought and under each of them I caused 23 eggs to be placed, and in the company of these persons I opened one every day so that we could see firstly the origin of the veins and secondly what organ is first formed in the animal". What follows is practically a repetition of the facts available in Aristotle, but described with much greater clearness than either

Aristotle or Aldrovandus had been able to bring to the matter. On the third day, he saw the *globulus sanguineus* which *in vitello manifeste pulsabat*, and so solved his first problem. He decides that the first organ to be formed is the heart, and quotes Lactantius' experiments. He explains the large size of the eye as due to the fact that the most complicated part of the body needs the longest time for its manufacture. He correctly describes the various membranes, and the *faeces subviridies* in the intestines at hatching. Once he contradicts Aristotle, maintaining that on the tenth day the body as a whole is larger than the head, and once he contradicts Albertus, denying that any yolk can be found in the stomach at hatching. He concludes his tractate by a succinct and clear account of the opinions of Aristotle and Hippocrates about embryonic development. His importance is that he drew the attention of scientific thinkers to the problems arising out of the hen's egg, and assisted in the formation of that iconographic phase in embryology which was later to find its climax in the plates of Fabricius, and its close in Harvey's *Exercitations*.

Gabriel Fallopius, who belongs to this time, must be mentioned as the discoverer of the organs which bear his name, but his services to embryology were only indirect. A. Benedictus, who was now growing old, and Caesar Cremonius, who was still young, may be remembered as the principal upholders of pure Aristotelianism at this time. Realdus Columbus also wrote on the embryo. B. Telesius, in his *De Natura Rerum* of 1565, studied the hen's egg and suggested that the parts of animals were formed by the pressure of the uterus acting as a mould: he was thus the middle term between Galen and Buffon.

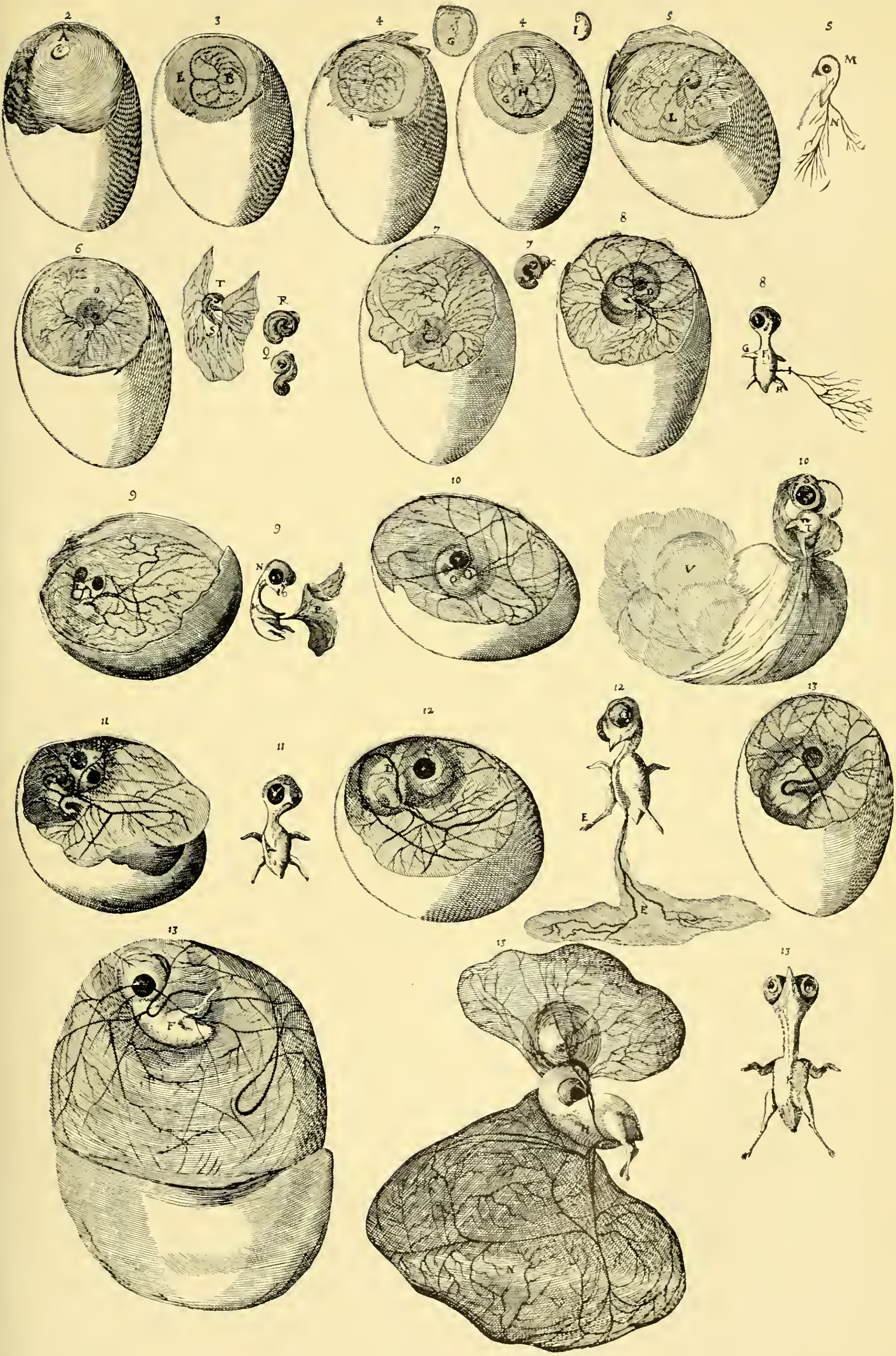
Julius Caesar Arantius has already been referred to. His *De Humano Foetu* was an important book, but, though it appeared in 1564, just at the time when the macro-iconographic school was at its height, it dealt with a rather different field and cannot be considered as a constituent of that group. He begins by relating that a pregnant woman was killed by an accident at Bologna a couple of years before, so that he had an opportunity of testing whether the opinions about certain points in generation, which he had formed on *a priori* grounds during the previous fifteen years, were true or not. In the first place, he found on dissection that the placenta was not cotyledonous, and he spoke thus of its formation: "Blood flows out from the spongy substance of the uterus and this blood growing in bulk forms a soft and fungus-like mass of flesh, rather like the substance of the spleen, which adheres to the surface of the uterus and transmits to the foetus in proportion as it grows the nourishment for it which reaches the uterus in the form of blood and spirits". Then, going on to discuss the functions of the *jecor uterinae*, as he calls the placenta (with what justice may be seen by turning to Section 8·5), he devotes a chapter to *De vasorum umbilicalium origine*, and, contradicting Hippocrates, Galen, Erasistratus, and Aetius, says that the maternal and foetal blood-vessels do not pass into each other by a free passage. "This is repugnant to sense", he writes, "and as may be seen by ocular inspection, these vessels do not reach the inner membrane of the uterus, for the substance of the placenta is placed between their ramifications and the proper substance of the womb." He was thus the first to maintain that the maternal and foetal circulations are separate, but he naturally did not, and could not, speak of circulations, since he lived before Harvey. Nor could he have satisfactorily proved his point with the means then at his command, and, as we shall see, it was to take another century before the proof was given. Apart from this valuable contribution to embryology, Arantius gave some admirable anatomical descriptions of the foetal membranes.

Hieronymus Fabricius ab Aquapendente, the pupil of Fallopius, has always been given an important place in the history of embryology by those who have written on him. As one comes upon him in the process of tracing out that history itself, however, he does not take such a high place. With the statement, for instance, that "Fabricius carried embryology far beyond where Coiter had left it and elevated it at one bound into an independent science" I find that I cannot agree. Embryologists who called themselves that and nothing else did not appear till the end of the eighteenth century, and it seems to me doubtful whether the anatomical advances in embryology made by Fabricius are not counterbalanced by the erroneous theories which he invented at the same time. His *De Formatione Ovi et Pulli pennatorum*, and his *De Formato Foetu* of 1604 show far more scholasticism and mere argumentativeness than is to be found in Coiter, and are remarkable for their bulk. Fabricius seems to have had a genius for exsuccous and formal discussions. He spends much time, for example, in taking up the problem of whether the yolk of the hen's egg is more earthy than the white, and looking at it from all possible angles. He disagrees at last with Aristotle and decides that the white is the more earthy. Bones, he says, are white, but also very earthy. The albumen is colder, stickier, and heavier than the yolk, "*sequitur, terrestrius esse*". And this particular example is the more flagrant because the actual matter of it is fundamentally physico-chemical. But, in addition, he introduced a number of grave errors and misleading theories into embryology, so that subsequently Harvey had to spend a large part of his time refuting them. Fabricius was, indeed, a good comparative anatomist, and it is upon that ground that he deserves praise: his plates, some of which are reproduced herewith, were far better than anything before and for a long time afterwards. He dissected embryos of man, rabbit, guinea-pig, mouse, dog, cat, sheep, pig, horse, ox, goat, deer, dogfish, and viper, a comparative study which had certainly never been made previously.

In his first tractate he begins by dealing with a question not unlike that of how the sardines got into the tin, i.e. how the contents got into the hard-shelled egg. He rejects Aristotle's idea that the egg is formed in the oviduct by a kind of umbilicus, and ascribes its growth there to transudation through the blood-vessels. He marks a definite advance upon Aristotle when he says that silkworms and other insects are born into their larval state from an egg, though he still terms the chrysalis an egg, and therefore holds that they are generated twice. Then follows his discussion of what part of the egg the chick comes from. The chalazae, he says, are not semen, for the semen is not present at all in the fertilised egg. His argument sounds peculiar when he says that both the white and yolk of the egg are the food of the embryo, for neither of them is absent at the end of incubation, therefore neither of them is its material. Hippocrates had said, "*ex luteo gigni, ex albo nutriri*"; Aristotle had said, "*ex albo fieri, ex luteo nutriri*". The latter was the view generally held in the sixteenth century, as may be gathered from Ambrosius Calepinus' dictionary, Scaliger's *Commentary on Aristotle*, and the treatise on the soul of Johannes Grammaticus.

Fabricius now says both nourish, neither makes. This distinction between food and building-materials seems to us unnecessary, but it had a great influence on later thought. Fabricius devotes much time to proving, as he thinks, that albumen and white are of the

PLATE V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ILLUSTRATIONS FROM FABRICIUS AB AQUAPENDENTE'S
DE FORMATIONE OVI ET PULLI, 1604

same nature, and adduces the fact that "in cooking the white hardens first, whether the egg be boiled or poached, but the yolk hardens also if the heat is more", comparing the heat of the kitchen to the innate heat of the chick. "But you will say", he goes on, "if the albumen and the yolk are the food of the chick in the egg, what then must we decide the material of the chick to be, since we have already said that the semen is not present in the eggs. You will find this material from an enumeration of the parts of the egg—there remains only the shell, the two membranes, and the chalazae;—nobody will assign the membranes or the shell as the material of the chick, therefore the chalazae alone are the fitting substance out of which it can be made." Having discovered this truth by the infallible processes of logic, Fabricius brings all kinds of arguments forward to support it; he adduces the three nodes in the chalazae as the precursors of brain, heart, and liver; tadpoles, he thinks, resemble significantly the chalazae, being "armless legless spines". The eyes are transparent, so are the chalazae, therefore the latter must give rise to the former. The liver is formed as soon as the heart but is practically invisible as it does not palpitate. One of his most gratuitous errors was the suggestion, now newly introduced, that the heart (and other organs) of the foetus has no proper function, no *munus publicum*, but beats only in order to preserve its own life. Then there is a considerable section called *De Ovorum utilitatibus*, which almost does for the hen's egg what Galen's *De Usu Partium* did for the human body, and in which such questions as Why the shell is hard and porous? and Why there are any membranes in the egg? are taken up and answered with an elaborate display of common sense. The influence of Galen is perceptible in a passage about a liver-like substance being formed if blood is freshly shed into hot water, in the usual terminology of formative faculties, and in the division of fleshes into white and red, though the former is not specifically derived from the semen nor the latter from the menstrual blood. The human placenta is described as cotyledonous, and needless confusion is caused by the doctrine that the "liquors, humours, or rather, excrements, around the foetus, are two in number, sweat and urine, the former in the amnios, the latter in the allantois". But the drawings and illustrations of Fabricius' work are beautiful and accurate—so much so, indeed, that it will always remain a mystery how the man who figured the early stages of the development of the chick as Fabricius did, showing the blood-vessels radiating from the minute heart, should have been able to propound the thesis that the chalazae were the material of the embryo.

The other biologist to whom Harvey was most indebted was Andreas Laurentius of Montpellier, whose *Historia Anatomica* (printed with his other works in 1628) contained a whole book (VIII) devoted to embryology, but which presents us with nothing except a commentary on Hippocrates and Aristotle. The only evidences of life are furnished by two polemics, one of which was against Simon Petreus of Paris, who had propounded some new views about the foetal circulation. Laurentius gave also a table showing the changes which occur in the heart and lungs of the foetus at birth.

It was about this time that the embryological observations of that many-sided genius, Hieronymus Cardanus, began to attract attention. His main thesis was that the limbs of the embryo were alone derived from the yolk, while the rest of the body came from the white. This was a well-meant attempt to mediate between the two traditions headed respectively by Aristotle and Hippocrates, but the arguments in support of it were not even remarkable for ingenuity. Constantinus Varolius treated of the formation of the embryo in a book which appeared in 1591, but very inadequately. He had certainly opened hen's eggs, and describes the fourth-day embryo as *forma minimi faseoli*. But nearly every one of his marginal headings begins with the word *Cur*, and this tells its own story, for the didactic style rarely hides genuine works of research. Johannes Fernelius, a rather earlier worker, in his *De Hominis Procreatione* followed Aristotle and Galen in nearly all particulars, and made no real contribution to embryology. On its practical obstetrical side, the sixteenth century produced some remarkable compilations of ancient gynaecological writings. The first of these was that of Caspar Wolf, which was published at Zürich in 1566, and, after having been enlarged by Caspar Bauhin in 1586, subsequently formed the backbone of the most important and famous one, namely, that of Israel Spach (Strassburg, 1597). Although these composite text-books represented no real embryological progress, they yet showed that great interest in development was alive, an interest which, though doubtless utilitarian in its origin, could hardly fail to lead to advances of a theoretical nature. (See Fig. 5.)

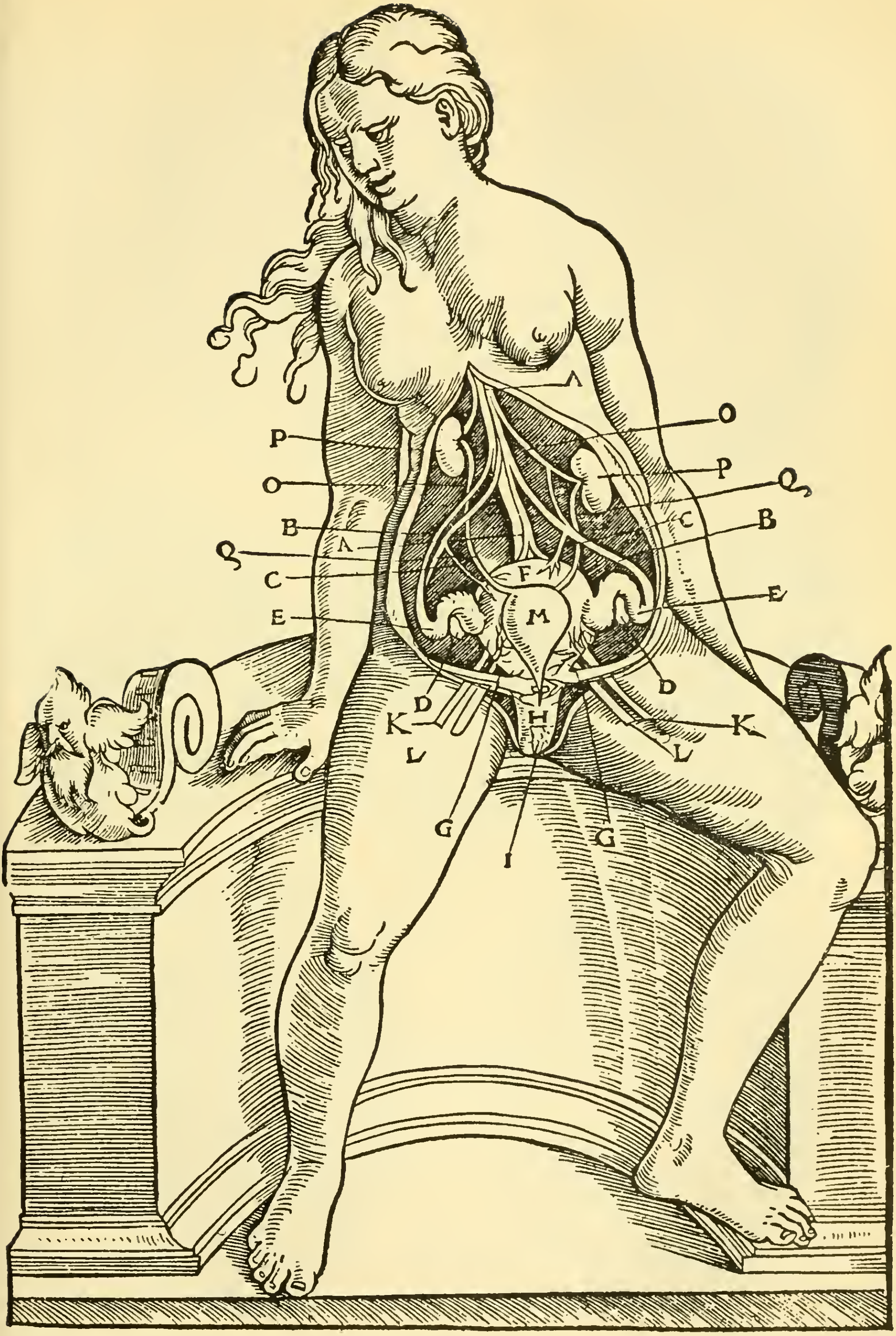
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Fig. 5. Illustration from W. H. Ryff's *Anatomia* of 1541.

The obstetrical literature intended for midwives is also of great interest. It was about this time that the first popular guides to their subject began to appear, founded not upon mere superstition and the remnants of ancient knowledge derived in roundabout fashion through Syriac and Arabic, but either upon a careful study of Galen and Aristotle, or upon the results of dissections and living speculation. The principal representative of the former class is that of Jacob Rueff, which appeared in 1554 and was called *De Conceptu et Generatione Hominis*. Although written in Latin, it was evidently a popular work, for the illustrations given in it are such as would naturally be incorporated in such a book. It is the illustrations which give it its importance, and I reproduce them in Fig. 6. I think they show very clearly what the general ideas were at this period about mammalian embryology, and thus afford us a precious insight into what was in the minds of such writers as Riolanus the elder, Mercurialis, Saxonia, Rondeletius, Venusti, Holler and Vallesius. There are many points which their expositions of foetal growth and development leave vague, and without Rueff it would be difficult or impossible to picture in what manner they imagined it to go on. Rueff's text follows Galen and Aristotle with fidelity, as does theirs—with the exception of a few minor ideas not quite consonant with this.

In (*a*) of Fig. 6 Rueff portrays the mixture of semen and menstrual blood in the womb, or, as he loosely refers to it, of both seeds, coagulating into a pink egg-shaped mass surrounded with a fine pellicle. (*b*) shows the same mass in the uterus and wrapped round with the three coats, amnion, chorion, and allantois—a lamentable but interesting misrepresentation of the facts. Then in (*c*) it is shown that upon the surface of the yolk-like mass of semen and blood appear "three tiny white points not unlike coagulated milk", these being the first origins of the liver, the heart, and the brain. Next (*d*) shows the first blood-vessels springing from the heart, four in number, and distributing themselves over the surface of the mass. It is plain that Rueff must either have opened hen's eggs himself and seen the early growth of the blastoderm or have been told about it by some observer such as Coiter or Aldrovandus. He could not have copied his pseudo-blastoderm pictures from their works, for in 1554 none of them had appeared, and, as far as I know, there were no similar illustrations in existence at that time.

After this point the pictures grow even more fanciful, and, in (*e*),

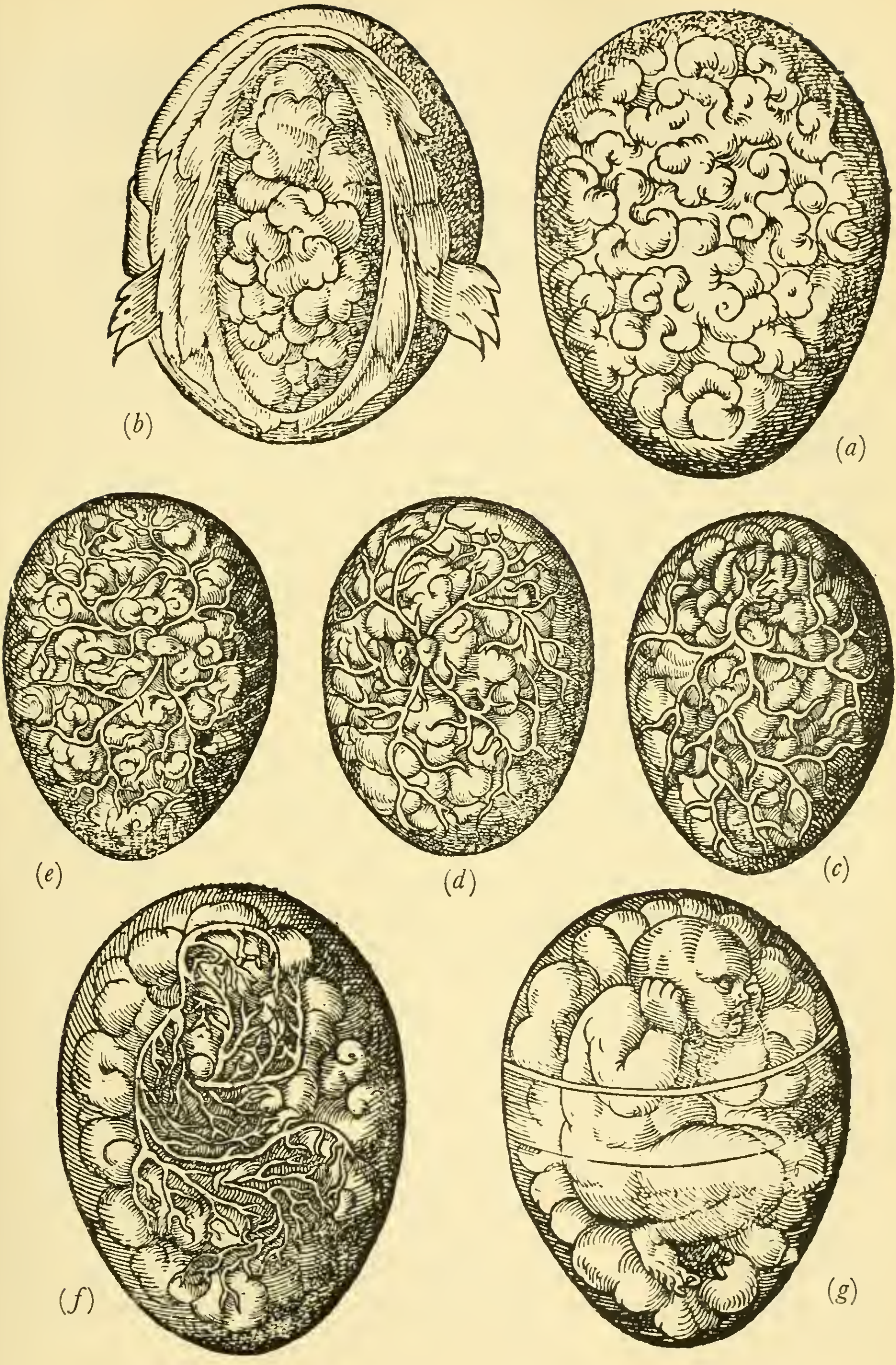


Fig. 6. Illustrations from Jacob Rueff's *De Conceptu et Generatione Hominis* of 1554 (arranged by Singer) showing the Aristotelian coagulum of blood and seed in the uterus.

the first outline of the cranium is seen taking shape in the upper part of the "egg". In (*f*) the blood-vessels have suddenly assumed the outline of a human being, and in (*g*) the finished product is seen. Rueff gives what seems to be a mnemonic in hexameters:

> iniectum semen, sex primis certe diebus
> est quasi lac: reliquisque novem sit sanguis; at inde
> consolidat duodena dies; bis nona deinceps
> effigiat; tempusque sequens producit ad ortum
> talis enim praedicto tempore figura consit.

Rueff gives some excellent diagrams of the foetus *in utero* with relation to the rest of the body, and the various positions which are familiar to obstetricians. His teratology is less happy, for he attributes the production of monsters to the direct action of God, though he does venture upon a few speculations concerning "corrupt seed". But his principal significance for this history is that, in his picture of the yolk-like mass of mixed semen and blood and the pseudo-blastoderm upon it, he throws a good light on the conceptions of the time.

Rueff's book was subsequently translated into English, and had many editions as *The Expert Midwife*.

The principal representative of the second class of popular books of this period is that of Euch. Rhodion, or Rösslein, which was translated into English, and published as his own work, by Thomas Raynold, "physition", in 1545, under the title of *The Byrth of Mankynde otherwyse named The Woman's Book* (cf. d'Arcy Power). It was the first book in the English language to contain copper engravings. They were variants of the traditional Soranus-Moschion figures. The Rösslein-Raynold book pays less attention to Galenic theory than does that of Rueff, and includes much better drawings of actual dissections. Another famous obstetrical book was that of Scipio Mercurius; for further information here see Spencer.

The minor embryologists of the sixteenth century included among them Ambroise Paré, the founder of modern surgery. His teaching on generation involved nothing original, but it seems to have been Galenism interpreted by a very intelligent and well-balanced, unspeculative mind. The three-bubble theory appears in him very clearly; thus, we read, "The seed boileth and fermenteth in the womb, and swelleth into three bubbles or bladders"—the brain, the liver, and the heart. Paré's illustrations are copied wholesale from Vesalius and Rueff, without acknowledgment. The last author to take the three-bubble theory quite seriously was A. Deusingius, who wrote in 1665, after Harvey. Others who deserve a mention, but no more, were Severinus Pinaeus, L. Bonaciolus and Felix Platter. None of them made any advance, and the illustrations of the former's *De Virginitatibus notis graviditate et partu* were almost ludicrous.

Hieronymus Capivaccius, F. Licetus, J. Costaeus and V. Cardelinus, who wrote in 1608, were the last true supporters of the ancient theories, such as that the male embryo was twice as hot and developed twice as quickly as the female.

SECTION 3

EMBRYOLOGY IN THE SEVENTEENTH AND EIGHTEENTH CENTURIES

3·1. The Opening Years of the Seventeenth Century

Æmilius Parisanus, a Venetian, now dealt with embryology in the fourth, fifth, and sixth books of his *De Subtilitate*. They were entitled as follows: "(4) Of the principles and first instruments of the soul and of innate heat, (5) Of the material of the embryo and of its efficient cause, (6) Of the part of the animal body which is first made, and of the mode and order of procreation". Parisanus is very wordy, but he has the merit of giving many quotations from the lesser known authors, and providing (as a rule) accurate references. He held that the spleen was formed in all development before the heart, and that neither heart nor lungs moved *in utero*. With regard to the controversy over the function of white and yolk, he was in agreement with Fabricius, but he firmly opposed the view that the chalazae were the first material of the chick, as much, it must be confessed, because of the opinion of Aristotle as from his own observation. Nevertheless, his own observations were noteworthy, and he will always be remembered for his discovery of the fact that the heart of the chick begins to beat some time before any red blood appears in it.

Parisanus was the last of the macro-iconographic group of sixteenth-century embryologists. Their labours established the fundamental morphological facts about the developing embryo; the first great step in the history of embryology. But there were numerous errors in their work, and Harvey, who occupies a terminal or boundary position, was destined to correct them. He marks the transition from the static to the dynamic conception of embryology, from the study of the embryo as a changing succession of shapes, to the study of it as a causally governed organisation of an initial physical complexity, in a word, from Coiter and Fabricius to Descartes and Mayow. Iconography did not die: on the contrary, the improvement of the microscope gave it new life, and the micro-iconographic school emerged with its principal glory, Malpighi.

Harvey sums up the work of the macro-iconographic period in the historical introduction contained in Ex. XIV of his *De Generatione Animalium*. I give it in full in the beautiful seventeenth century English into which Harvey's Latin was translated under his guidance by the physician, Martin Llewellyn.

"We have already discovered the Formation, and Generation of the Egge; it remains that we now deliver our Observations, concerning the Procreation of the Chicken out of the Egge. An undertaking equally difficult, usefull, and pleasant as the former. For Nature's Rudiments and Attempts are involved in obscurity and deep night, and so perplext with subtilties, that they delude the most piercing wit, as well as the sharpest eye. Nor can we easier discover the secret recesses, and dark principles of Generation than the method of the fabrick and composure of the whole world. In this reciprocal interchange of Generation and Corruption consists the Æternity and Duration of mortal creatures. And as the Rising and Setting of the Sun, doth by continued revolutions complete and perfect Time; so doth the alternative vicissitude of Individuums, by a constant repetition of the same species, perpetuate the continuance of fading things.

"Those Authors which have delivered any thing touching this subject, do for the most part tread a several path, for having their Judgements prepossessed with their own private opinions, they proceed to erect and fashion principles proportionable to them.

"Aristotle of old, and Hieronymus Fabricius of late, have written so accurately concerning the Formation and Generation of the Foetus out of the Egge, that they seem to have left little to the industry of Posterity. And yet Ulysses Aldrovandus hath undertaken the description of the Pullulation or Formation of the chicken out of the Egge, out of his own Observations; wherein he seems rather to have directed and guided his thoughts by the Authority of Aristotle, than by his own experience.

"For Volcherus Coiter, living at Bononia at the same time did by the advice of the said Aldrovandus (whom he calls Tutor) dayly employ himself in the opening of Egges sat upon by the Hen, and hath discovered many things truer than Aldrovandus himself, of which he also could not be ignorant. Likewise Æmilius Parisanus (a Venetian Doctor) despising other mens opinions hath fancied A new procreation of the Chicken out of the Egge.

"But because somethings, (according to our experience) and those of great moment and consequence, are much otherwise than hath been yet delivered, I shall declare to you what dayly progress is made in the egge, and what parts are altered, especially about the first dayes of Incubation; at which time all things are most intricate, confused, and hard to observe, and about which authors do chiefly stickle for their own observations, which they accomodate rather to their own preconceived perswasions (which they have entertained concerning the Material and Efficient Causes of the generation of Animals) than to truth herself.

"Aldrovandus, partaking of the same error with Aristotle, saith (which none but a blind man can subscribe to) that the Yolk doth in the first dayes, arise to the Acute Angle of the Egge; and thinks the Grandines to be the Seed of the Cock; and that the Pullus is framed out of them, but nourished as well by the yolk as the white; which is clean contrary to Aristotle's opinion, who conceived the Grandines to conduce nothing to the fecundity of the egge. Volcherus Coiter delivers truer things, and more consonant to Autopsie, yet his three Globuli are meer fables. Nor did he rightly consider the principle from whence the Foetus is derived in the Egg. Hieronymus Fabricius indeed contends, that the Grandines are not the seed of the cock, and yet he will have the body of the Chicken to be framed out of them (as out of its first matter) being made fruitful by the seed of the cock. He likewise saw the Original of the Chicken in the Egge; namely the Macula, or Cicatricula annexed to the membrane of the Yolke but conceived it to be onely a Relique of the stalk broken off, and an in-firmity of blemish onely of the Egge, and not a principle part of it. Parisanus hath plentifully confuted Fabricius his opinion concerning the Chalazae or Grandines, and yet himself is evidently at a loss in some certaine circles and points of the Principle parts of the Foetus (namely the Liver and the Heart) and seems to have observed a Principium or first Principle of the Foetus, but not to have known which it was, in that he saith, that the Punctum Album in the Middle of the Circles is the Cocks Seed out of which the Chicken is made. So that it comes to pass that while each of them desire to reduce the manner of the Formation of the Chicken out of the Egge to their own opinions they are all wide from the mark."

Before discussing how Harvey put them right, however, there are a number of other matters to be mentioned. Parisanus' work was published in 1623, and twenty-five years were to elapse before Harvey's *Exercitations* were to be put before the learned world by George Ent. In that time not a few events of importance for the history of embryology took place.

It will be convenient to speak first of Adrianus Spigelius, whose *De Formato Foetu* appeared in 1631. In this book the plates of the gravid uterus which had been prepared some years before for Julius Casserius were now published. They had more influence than Spigelius' text, perhaps, in contributing to the permanent fame of his book.

He gives for the most part straightforward anatomical descriptions, but he returns to the notion of a cotyledonous placenta in man, and he combats Arantius' opinions about the placenta. Arantius had said that the function of the *jecor uterinae* was to purify the blood-supply to the foetus, a thoroughly modern idea, but Spigelius opposes this on two grounds, firstly, because the foetus has its own organs for purifying blood, and secondly, because, if Arantius was right, the placenta would always be as red as blood, but this is not the case in such animals as the sheep. Spigelius himself thought that the placenta was for the purpose of preventing severe loss of blood at birth, as would be the case if the embryo was joined to the mother with only one big vessel and not a great many little ones.

However, Spigelius upholds the view, taken by Rufus of Ephesus and by Vesalius, that the allantois contains the foetal urine, which has to be separated from the amniotic liquid in which the embryo is, because it would corrode the embryonic skin (*ne cuti tenellae aliquod damnum urinae acrimonia inferret*). This passage is interesting, as showing biochemical rudiments. The first discussion of the *vernix caseosa*, or *sordes*, as he calls it, appears in Spigelius, who, however, hazards no guess as to its nature. He is happy in his refutation of Laurentius, who had affirmed that the foetal heart did not beat *in utero*, and he shows some advance on all previous writers save Arantius in declaring that the umbilical vessels take vital spirits away from the foetal heart, not exclusively to it. He gave, moreover, the first denial of the presence of a nerve in the umbilical cord, and also made the first observation of the occurrence of milk in foetal breasts at birth (for the endocrinological explanation of this see Section 15). Finally, he abolished at last the notion that the meconium in the foetal intestines argued eating *in utero* on the part of the embryo.

Riolanus the younger, the correspondent and almost exactly the contemporary of Harvey, was Professor in Paris and published his *Anthropographia* in 1618. As he was a keen advocate of the ancient views, his section on the formation of the foetus has little importance. Yet it contains the first known instance of the use of the lens in embryology, the germ of that powerful instrument which was to lead in due course to so many discoveries. "In aborted embryos", said Riolanus, "the structure is damaged and can often not be properly seen, even when you make use of lenses [*conspicilia*] which make objects so much bigger and more complicated than they ordinarily seem."

The *De Formatrice Foetus* of Thomas Fienus, Professor at Louvain and a friend of Gassendi, published in 1620, is interesting because it is the middle term between Aristotle and Driesch. As the title-page informs us, he sets out to demonstrate that the rational soul is infused into the human embryo on the third day after conception. This by itself would not be very attractive, but the most cursory inspection shows that Fienus' interests were not at all theological. He divides the book up into seven main questions. (1) What is the efficient cause of embryogeny? He concludes that it is neither God, nor Intelligence, nor *anima mundi* (influence of Neo-platonism here as on Galileo). (2) Is it in the uterus or in the seed? In the latter, says Fienus, adding a list of authorities who agree with this view—Haly-Abbas, Gaietanus, Zonzinas, Turisanus, Fernelius, Vallesius, Peramatus, Saxonia, Carrerius, Zegarra, Mercurialis, Massaria, and Archangelus, "*solus Fabio Pacio utero imprudenter adscribit*" (!). (3) Is it heat? Fienus nearly decided that it was, and, if he had done so, would have shown a modern mind, but no, he gave his opinion against it, saying, "the process (of development) is so divine and wonderful that it would be ridiculous to ascribe it to heat, a mere naked and simple quality". After weighing various other alternatives in questions (4), (5) and (6), he asks whether it is "*anima seminis post conceptum adveniens*" (7), and concludes that it is. It is here that he becomes really interesting, for he quotes with approval certain writers, e.g. Alexander Aphrodisias (*Organicum corpus esse organicum ab anima et anima praeexistere organizationi*), Themistius (*Anima fabricatur architecturaque sibi domicilium et accommodatum instrumentum*) and Marsilio Ficino in his commentary on Plato's *Timaeus* (*Priusquam adultum sit corpus, anima tota in illius fabrica occupatur*), and then maintains with them that the soul is the principle which organises the body from within, arranging an organ for each of its faculties and preparing a residence for itself, not merely allowing itself to be breathed into a being which has already organised itself. "The conformation of the foetus is a vital, not a natural, action", he says. He develops this idea in the remainder of the book; according to him, the seed first coagulates the menstrual blood into an amorphous cake, taking three days to do so, after which, the rational (not vegetative or sensitive) soul (entelechy), which has entered the uterus with the seed, finding a suitable mass of shapeless material, enters into it and begins to give it a shape. Fienus was attacked by several writers, and published a defence of his views.

Later writers on the same subject included Fidelis, Teichmeyer, Albertus, de Reies, Torreblanca and de Mendoza. The Spanish influence here is perhaps significant. Hieronymus Florentinus, who adopted the same standpoint as Fienus in 1658 was forced to recant it.

In 1625 Joseph de Aromatari, a Venetian, included in his epistle on plants the first definite statement of the preformationist theory since Seneca, but he did not develop the idea. He had noted that in bulbs and some seeds the rudiments of many parts of the adult plant can be seen even without glass or microscope, and this led him to suggest that probably in all animals as well as plants a similar thing was true. "And as for the eggs of fowls", he said, "I think the embryo is already roughly sketched out in the egg before being formed at all by the hen [*quod attinet ad ova gallinarum, existimamus quidem pullum in ovo delineatum esse, antequam formatur a gallina*]." This suggestion did not begin to bear its malignant fruits till the time of Swammerdam and Malpighi.

Johannes Sinibaldi's *Geneanthropia* might be mentioned as belonging to this time. It was a compilation of facts relating to the generation of man, but it expressly excluded from its field any discussion of the embryo. It is no more important for our subject than the queer *Ovi Encomium* of Erycius Puteanus, another of Gassendi's friends, which has already been referred to (p. 8).

3·2. Kenelm Digby and Nathaniel Highmore

Much more significant was the controversy between Sir Kenelm Digby and Nathaniel Highmore. In 1644, Sir Kenelm, whose intriguing personality will be sufficiently familiar to anyone even slightly acquainted with seventeenth-century England, and whose biographic details may be found in John Aubrey, published a work with the following title: *Two treatises, in the one of which, The Nature of Bodies, in the other, The Nature of Man's Soule is looked into, in way of discovery of the Immortality of Reasonable Soules.* It was inscribed in a charming dedication to his son, and consisted, in brief, of a survey of the whole realms of metaphysics, physics, and biology from a very individual point of view.

One of Sir Kenelm's principal objects in writing was apparently to attack the old terminology of "qualities" in physics and "faculties" in biology. To say, as contemporary reasoning did, that bodies were red or blue because they possessed a quality of redness or blueness which caused them to appear red or blue to us, or again, to say that the heart beat because it was informed by a sphygmic faculty, or, to take the famous example, that opium sent people to sleep because it contained in it a dormitive virtue, appeared mere nonsense and word-spinning to Digby, "the last refuge of ignorant men, who not knowing what to say, and yet presuming to say something, do often fall upon such expressions".

Digby, like Galileo and Hobbes, wished to explain all phenomena by reference to two "virtues" only, those of rarity and density, "working by means of locall Motion". Chapters twenty-three, twenty-four, and twenty-five contain his opinions and experiments in embryology. He begins by opening the question of epigenesis or preformation, practically for the first time since Albert the Great. "Our main question shall be", he says, "whether they be framed entirely at once, or successively, one part after another? And if this latter way, which part first?" He declares for epigenesis, but after a manner of his own, refuting "the opinion of those who hold that everything containeth formally all things". "Why should not the parts be made in generation", he asks, "of a matter like to that which maketh them in nutrition? If they be augmented by one kind of juyce that after severall changes turneth at the length into flesh and bone; and into every sort of mixed body or similar part whereof the sensitive creature is compounded, and that joyneth itself to what it findeth there already made, why should not the same juyce with the same progresse of heat and moisture, and other due temperaments, be converted at the first into flesh and bone though none be formerly there to joyn it self unto?" He gives a clearly deterministic account of development. "Take a bean, or any other seed and put it in the earth, and let water fall upon it; can it then choose but that the bean must swell? The bean swelling, can it choose but break the skin? The skin broken, can it choose (by reason of the heat that is in it) but push out more matter, and do that action which we may call germinating? Can these germs choose but pierce the earth in small strings, as they are able to make their way?...Thus by drawing the thrid carefully along through your fingers, and staying at every knot to examine how it is tyed; you see that this difficult progresse of the generation of living creatures is obvious enough to be comprehended and the steps of it set down; if one would but take the paines and afford the time that is necessary to note diligently all the circumstances in every change of it....Now if all this orderly succession of mutations be necessarily made in a bean, by force of sundry circumstances and externall accidents; why may it not be conceived that the like is also done in sensible creatures, but in a more perfect manner, they being perfecter substances? Surely the progresse we have set down is much more reasonable than to conceive that in the seed of the male there is already in act, the substance of flesh, bone, sinews, and veins, and the rest of those severall similar parts which are found in the body of an animall, and that they are but extended to their due magnitude by the humidity drawn from the mother, without receiving any substantiall mutation from what they were originally in the seed. Let us then confidently conclude, that all generation is made of a fitting, but remote, homogeneall compounded substance upon which outward Agents, working in the due course of Nature, do change it into another substance, quite different from the first, and do make it lesse homogeneall than the first was. And other circumstances and agents do change this second into a third, that third, into a fourth; and so onwards, by successive mutations that still make every new thing become lesse homogeneall than the former was, according to the nature of heat, mingling more and more different bodies together, untill that substance bee produced which we consider the period of all these mutations." This passage is indeed admirable, and well expresses the most modern conception of embryonic development, that of the ovum as a physico-chemical system, containing within itself only to a slight and varying degree any localisation answering to the localisation of the adult, and ready to change itself, once the appropriate stimulus has been received, into the completed embryo by the actions and reactions of its own constituents on the one hand and the influence of the fitting factors of the environment upon the other. Digby has not received his due in the past; he stands to embryology as an exact science, much in the same relationship as Bacon to science as a whole.

"Generation is not made", he says, "by aggregation of like parts to presupposed like ones; nor by a specificall worker within; but by the compounding of a seminary matter with the juice which accrueth to it from without and with the steams of circumstant bodies, which by an ordinary course of nature are regularly imbibed in it by degrees and which at every degree doe change it into a different thing..." (see p. 317). "Therefore to satisfie ourselves herein, it were well we made our remarks on some creatures that might be continually in our power to observe in them the course of nature every day and hour. Sir Iohn Heydon, the Lieutenant of his Majesties Ordnance (that generous and knowing Gentleman, and consummate Souldier both in theory and practice) was the first that instructed me how to do this, by means of a furnace so made as to imitate the warmth of a sitting hen. In which you may lay severall eggs to hatch, and by breaking them at severall ages you may distinctly observe every hourly mutation in them if you please." Sir Kenelm then goes on to describe the events that take place in the incubating egg, which he does very accurately, though briefly. In vivipara, he says, the like experiments have been made, and the like conclusions come to by "that learned and exact searcher into nature, Doctor Harvey"—these he must have learnt of by word of mouth, for Harvey's book had not at that time been published. As regards heredity, he adopts a pure theory of pangenesis, and has more to say about it than any other writer of his time. He is sure that the heart is first formed both in ovipara and vivipara, "whose motion and manner of working evidently appears in the twinckling of the first red spot (which is the first change) in the egge".

Sir Kenelm Digby not only anticipated the outlook of the physico-chemical embryologist, but he also foreshadowed with considerable accuracy Wilhelm Roux's definition of interim embryological laws. "Out of our short survey", he says, "of which (anserable to our weak talents, and slender experience) I perswade myselfe it appeareth evidently enough that to effect this worke of generation there needeth not to be supposed a forming virtue or *Vis Formatrix* of an unknown power and operation, as those that consider things suddenly and in grosse do use to put. Yet in discourse, for conveniency and shortnesse of expression we shall not quite banish that terme from all commerce with us; so that what we mean by it be rightly understood, which is the complex assemblement, or chain of all the causes, that concur to produce this effect, as they are set on foot to this end by the great Architect and Moderatour of them, God Almighty, whose instrument Nature is: that is, the same thing, or rather the same things so ordered as we have declared, but expressed and comprized under another name." Thus Sir Kenelm admits that it is allowable to speak of the "complex assemblement" of causes, as if it were one formative virtue, and this corresponds to Roux's "secondary components" or interim embryological laws. But that the portmanteau generalisations can be resolved into ultimate physico-chemical processes, Digby both believes and spends two entire chapters in trying to show. Digby has been one of the two seventeenth-century Englishmen most underestimated in the history of biology, but his place is in reality a very high one. How far he was in advance of his time may be gauged from the work of his contemporary Sperlingen, whose book of 1641 was thoroughly scholastic and retrograde.

His *Treatise on Bodies* evoked several answers. Undoubtedly the most interesting from the progressive side was that of Nathaniel Highmore, who will always be well remembered in embryological history. Highmore's *The History of Generation* came out in 1651, so that Harvey must have known of it, and it is one of the puzzles of this period why Harvey did not make any mention of it in his work, especially as J. D. Horst in a letter to Harvey refers to Highmore as his pupil. Harvey replying in 1655 said he had not seen Highmore for seven years. Highmore's title-page expressly states that his book is an answer to the opinions of Sir Kenelm Digby. But before discussing in what the answer consisted, we may look at the plate which is bound in immediately after the dedication (to Robert Boyle). It is interesting in that it shows again the idea initiated by Leonardo, namely, that all growing things, plants as well as animals, have an umbilical cord, and in that the drawings of the chick embryos and eggs are more quaint than accurate (Plate VI).

Highmore first describes the Aristotelian doctrine of form and matter, and then censures both it and the extensions of it with their "qualities", etc., much as Digby himself had done. "Some of our later philosophers have showed us that those forms w^{ch} they thought and taught to bee but potentially in the matter, are there actually subsisting though till they have acquired fitting organs, they manifest not themselves. And that the effects which were done before their manifestation (as the forming and fashioning of the parts wherein they are to operate) can rise from nothing else than from the Soul itselfe. This likewise I shall leave to the Readers enquiry, and shall follow that other way of introducing Forms, and Generation of creatures (as well animals as vegetables) which gives Fortune and Chance the preheminency in that work." He then describes Sir Kenelm's opinions, quoting from him in detail, and dissents from them mainly on the ground that they do not sufficiently account for embryogeny, as it were, from a technical point of view. That they subvert the "antique principals of philosophy" does not worry Highmore, but in his view their detailed mechanisms do not explain the facts, a much more serious drawback. Highmore is himself by way of being an Atomist, and it is because embryology was first treated by him from an atomistic standpoint that he derives his importance. "The blood, that all parts may be irrigated with its benigne moisture, is forc'd by several channels to run through every region and part of the body; by which meanes every part out of that stream selects those atomes which they finde to be cognate to themselves. Amongst which the Testicles abstract some spiritual atomes belonging to every part, which had they not here been anticipated, should have been attracted to those parts, to which properly they did belong for nourishment....These particles passing through the body of the Testicles, and being in this Athanor cohobated and reposited into a tenacious matter, at last passe through infinite Meanders through certain vessels, in which it undergoes another digestion and pelicanizing." Highmore objects, therefore, more to Digby's theory of pangenesis than to his description of embryogenesis. He goes on to give a long description of the development of the chick in the egg, mentioning in passing that the albumen corresponds to the semen and the blood of vivipara and the yolk to their milk. "Fabritius, who hath taken a great deal of pains in dissections... supposes the chick to be formed from the chalazae, that part which by our Women is called the treddle. But this likewise is false, for then every egge should produce 2 chickens, there being one treddle

PLATE VI

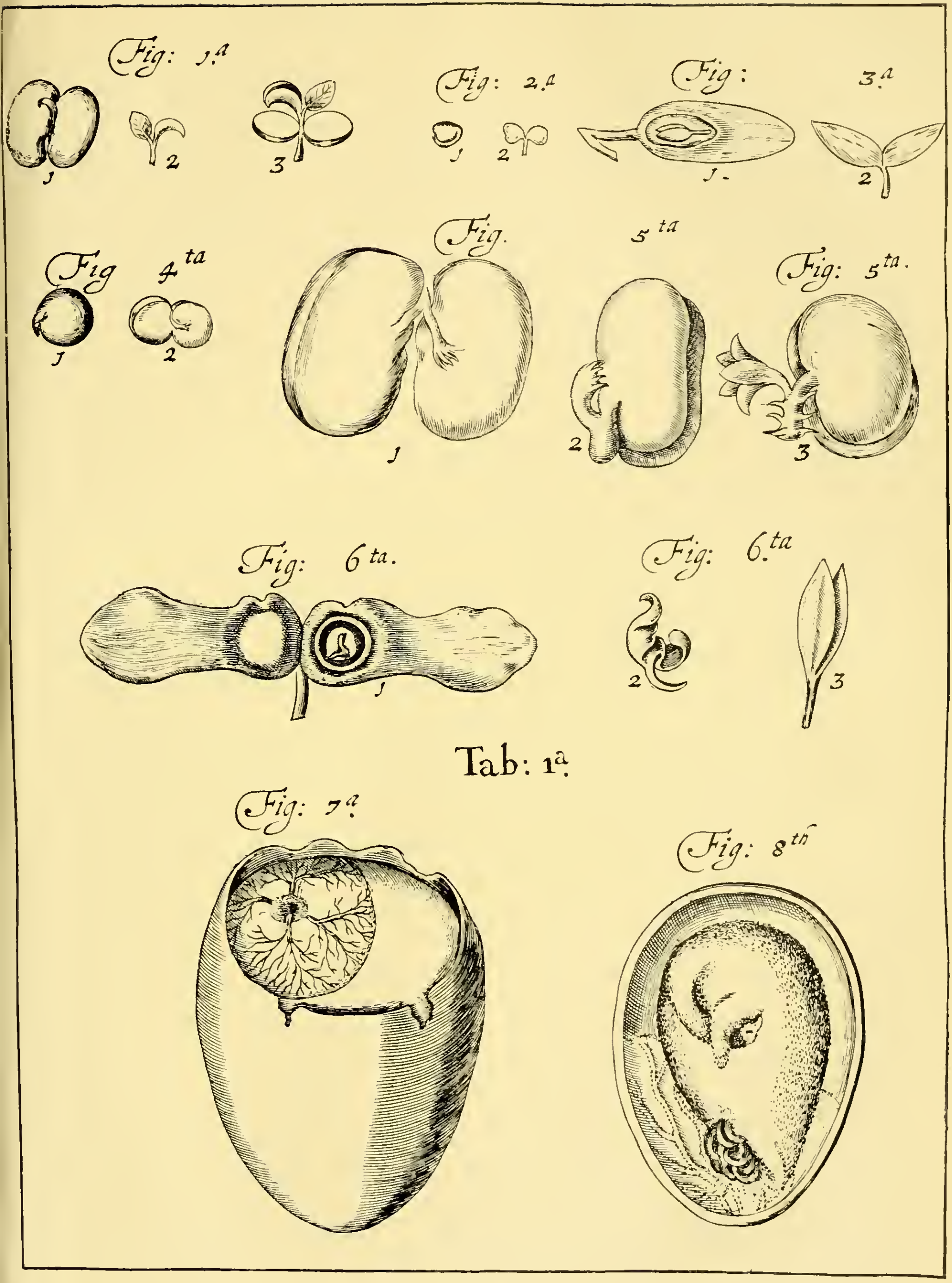


Fig: 1.a
1
2
3
Fig: 2.a
1
2
Fig: 3.a
1.
2
Fig 4ta
1
2
Fig. 5ta
1
2
Fig: 5ta.
3
Fig: 6ta.
1
Fig: 6ta
2
3
Tab: 1a.
Fig: 7a
Fig: 8th

ILLUSTRATION FROM NATHANIEL HIGHMORE'S *HISTORY OF GENERATION*, 1651

at each end of the egg, which serve for no other end than for ligaments to contain the yolk in an equilibrium, that it might not by every moving of the egg be shakt, broke, and confused with the white." Highmore was the first to draw attention to the increase of brittleness which takes place in the egg-shell during incubation, and he holds still to the Epicurean view that the female produces a kind of seed, though he thinks that the chick embryo is nourished in the early stages by the amniotic liquid.

Perhaps the most interesting reply to Digby from the traditional angle was that of Alexander Ross. In his *Philosophicall Touchstone* he upheld the Galenic view that the liver must be first formed in generation, for the nourishment is in the blood and the blood requires a liver to make it: ergo, the liver must be the earliest organ. Such arguments could dispense with observations. Ross also mentions Digby's suggestion that the "formative virtue" was only a bundle of natural causes, but he claims that the notion was an old one in school-philosophy, being included in the phrase *causa causae, causa causati*.

3·3. Thomas Browne and the Beginnings of Chemical Embryology

There are references to embryology in Sir Thomas Browne's *Pseudodoxia Epidemica, or Inquiries into very many vulgar Tenents and commonly received Truths*, which was published at this time. The twenty-eighth chapter of the third book contains a number of difficult problems in the embryology of the period, in most cases stated without any solution. "That a chicken is formed out of the yolk of the Egg was the opinion of some Ancient Philosophers. Whether it be not the nutrient of the Pullet may also be considered; since umbilical vessels are carried into it, since much of the yolk remaineth after the chicken is formed, since in a chicken newly hatched, the stomack is tincted yellow and the belly full of yelk which is drawn at the navel or vessels towards the vent, as may be discerned in chickens a day or two before exclusion. Whether the chicken be made out of the white, or that be not also its aliment, is likewise very questionable, since an umbilical vessel is derived unto it, since after the formation and perfect shape of the chicken, much of the white remaineth. Whether it be not made out of the grando, gallature, germ, or tred of the egg, as Aquapendente informeth us, seemed to many of doubt; for at the blunter end it is not discovered after the chicken is formed, by this also the white and the yelk are continued whereby it may conveniently receive its nutriment from them both....But these at last and how in the Cicatricula or little pale circle formation first beginneth, how the Grando or tredle, are but the poles and establishing particles of the tender membrans firmly conserving the floating parts in their proper places, with many observables, that ocular Philosopher and singular discloser of truth, Dr Harvey hath discovered, in that excellent discourse of generation, so strongly erected upon the two great pillars of truth, Experience, and Reason.

"That the sex is discernable from the figure of eggs, or that cocks or hens proceed from long or round ones, experiment will easily frustrate....Why the hen hatcheth not the egg in her belly? Why the egg is thinner at one extream? Why there is some cavity or emptiness at the blunter end? Why we open them at that part? Why the greater end is first excluded [cf. p. 233]? Why some eggs are all red, as the Kestrils, some only red at one end, as those of kites and buzzards? Why some eggs are not oval but round, as those of fishes? etc. are problems whose decisions would too much enlarge this discourse." And elsewhere, "That (saith Aristotle) which is not watery and improlifical will not conglaciate; which perhaps must not be taken strictly, but in the germ and spirited particles; for Eggs, I observe, will freeze, in the albuginous part thereof". Again, "They who hold that the egg was before the bird, prevent this doubt in many other animals, which also extendeth unto them; for birds are nourished by umbilical vessels and the navel is manifest sometimes a day or two after exclusion....The same is made out in the eggs of snakes, and is not improbable in the generation of Porwiggles or Tadpoles, and may also be true in some vermiparous exclusions, although (as we have observed in the daily progress of some) the whole Magot is little enough to make a fly without any part remaining....The vitreous or glassie flegm of white of egg will thus extinguish a coal."

These citations show Sir Thomas to have been more than simply the supreme artist in English prose which is his common title to remembrance. In picking his way carefully among the doubtful points and difficult problems which previous embryologists had propounded but not answered, he usually managed to give the right answer to each. But in addition to this, he was also an experimentalist, he had made both anatomical and physical experiments on eggs, and he was prepared to put any disputed point to the test of "ocular aspection", if this could be done. His experimental contributions to embryology come out more clearly in his *Commonplace Books* which were published by Wilkin in 1836.

"Runnet beat up with the whites of eggs seems to perform nothing, nor will it well incorporate, without so much heat as will harden the egg....Eggs seem to contain within themselves their own coagulum, evidenced upon incubation, which makes incrassation of parts before very fluid....Rotten eggs will not be made hard by incubation or decoction, as being destitute of that spirit or having the same vitiated....They will be made hard in oil but not so easily in vinegar which by the attenuating quality keeps them longer from concoction, for infused in vinegar they lose the shell and grow big and much heavier then before....In the ovary or second cell of the matrix the white comes upon the yolk, and in the later and lower part, the shell is made or manifested. Try if the same parts will give any coagulation unto milk. Whether will the ovary best?...The whites of eggs drenched in saltpeter will shoot forth a long and hairy saltpeter and the egg become of a hard substance. Even in the whole egg there seems a great nitrosity, for it is very cold and especially that which is without a shell (as some are laid by fat hens) or such as are found in the egg poke or lowest part of the matrix, if an hen be killed a day or two before she layeth....Difference between the sperm of frogs and eggs, spawn though long boiled, would not grow thick and coagulate. In the eggs of skates or thornbacks the yolk coagulates upon long docoction, not the greatest part of the white.... In spawn of frogs the little black specks will concrete though not the other....In eggs we observe the white will totally freeze, the yolk, with the same degree of cold will grow thick and clammy like the gum of trees, but the sperm or tread hold its former body, the white growing stiff that is nearest to it."

The only conclusion that can be drawn from these remarkable observations is that it was in the "elaboratory" in Sir Thomas' house at Norwich that the first experiments in chemical embryology were undertaken. His significance in this connection has so far been quite overlooked, and it is time to recognise that his originality and genius in this field shows itself to be hardly less remarkable than in so many others. To have occupied himself with the chemical properties of those substances which afford the raw material of development was a great step for those times, but it was not until some twenty-five years later that Walter Needham carried this new interest into the mammalian domain, and made chemical experiments there.

3·4. William Harvey

The Latin edition of William Harvey's book on the generation of animals appeared in 1651, and the English in 1653. The frontispiece of the former which is reproduced as the frontispiece of this book is a very noteworthy picture, and derives a special interest from the fact that on the egg which Zeus holds in his hands is written, "*Ex ovo omnia*",—a conception which Harvey is continually expounding (see especially the chapter, "That an egg is the common Original of all animals"), but which he never puts into epigrammatic form in his text, so that the saying, *omne vivum ex ovo*, often attributed to him, is only obliquely his.

The *De Generatione Animalium* was written at different times during his life, and not collected together for publication until George Ent, of the College of Physicians, persuaded Harvey to give it forth about 1650. As early as 1625 Harvey was studying the phenomena of embryology, as is shown among other evidences by a passage in his book where he says, "Our late Sovereign King Charles, so soon as he was become a man, was wont for Recreation and Health sake, to hunt almost every week, especially the Buck and Doe, no Prince in Europe having greater store, whether wandring at liberty in the Woods and Forrests or inclosed and kept up in Parkes and Chaces. In the three summer moneths the Buck and the Stagge being then fat and in season were his game, and the Doe and Hind in the Autumme and Winter so long as the three seasonable moneths continued. Hereupon I had a daily opportunity of dissecting them and of making inspection and observation of all their parts, which liberty I chiefly made use of in order to the genital parts". Nor was Harvey less diligent in examining the generation of ovipara. John Aubrey, in his *Brief Lives*, says, "I first sawe Doctor Harvey at Oxford in 1642 after Edgehill fight, but I was then too young to be acquainted with so great a Doctor. I remember that he came often to Trin. Coll. to one George Bathurst, B.D. who kept a hen in his chamber to hatch egges, which they did dayly open to discerne the progress and way of generation". Aubrey mentions a conversation he had with a sow-gelder, a countryman of little learning, but much practical experience and wisdom, who told him that he had met Dr Harvey, who had conversed with him for two or three hours, and "if he had been", the man remarked, "as stiff as some of our starched and formall doctors, he had known no more than they". Harvey seems also to have learnt all he could from the keepers of King Charles' forests, as several passages in his book show. Nor was the King's own interest lacking. "I saw long since a foetus", he says, "the magnitude of a peasecod cut out of the uterus of a doe, which was complete in all its members & I showed this pretty spectacle to our late King and Queen. It did swim, trim and perfect, in such a kinde of white, most transparent and crystalline moysture (as if it had been treasured up in some most clear glassie receptacle) about the bignesse of a pigeon's egge, and was invested with its proper coat." And, again—"My Royal Master, whose Physitian I was, was himself much delighted in this kinde of curiosity, being many times pleased to be an eye-witness, and to assert my new inventions".

Harvey's book is composed of seventy-two exercitations, which may be divided up for convenience into five divisions. In Nos. 1 to 10 he speaks of the anatomy and physiology of the genital organs of the fowl, and the manner of production of eggs. Nos. 11 to 13 and also Nos. 23 and 36 deal with the hen's egg in detail, describing its parts and their uses, while in Nos. 14 to 23 the process of the "generation of the foetus out of the hen egge" is described. The greater part of the book, comprising Nos. 25 to 62, as well as Nos. 71 and 72, is theoretical, and treats of the embryological theories held by Aristotle on the one hand, and the physicians, following Galen, on the other, instead of which it propounds new views upon the subject. Finally, Nos. 63 to 70, as well as the two appendices or "particular discourses", are concerned with embryogenesis in viviparous animals, especially in hinds and does.

It will be best to refer to certain details and main points of interest in Harvey's discussions, before trying to assess his principal contributions to the science as a whole. Harvey is the first, since Aristotle, to refer to the "white yolk" of birds. "For between the yolk", he says, "which is yet in the cluster and that which is in the midst of the eg when it is perfected this is the difference in chief, that though the former be yellowish in colour and in appearance, yet its consistence representeth rather the white, and being sodden, thickeneth like it, growing compact and viscous and may be cut into slices. But the yolk of a perfect egge being boiled groweth friable and of a more earthy consistence, not thick and glutinous like the white." All of Harvey's observations on the formation of the egg in the oviduct contained in this chapter are interesting, and may with advantage be compared with the studies of Riddle upon the same subject, where the chemical explanation will be found for many of Harvey's simple observations. Harvey's controversy with Fabricius on the question of whether the egg is produced with a hard shell or only acquires its external hardness upon standing in the air, which follows immediately on the above citation, is interesting. "Fabricius seemeth to me to be in errour, for though I was never so good at slight of hand to surprise an egge in the very laying, and so make discovery whether it was soft or hard, yet this I confidently pronounce that the shell is compounded within the womb of a substance there at hand for the purpose, and that it is framed in the same manner as the other parts of the egg are by the plastick faculty, and the rather, because I have seen an exceeding small egge which had a shell of its own and yet was contained within another egge, greater and fairer than it, which egge had a shell too."

Harvey was the first to note that the white of the hen's egg is heterogeneous, in the sense that part of it is much more liquid than the rest, and that the more viscous part seems to be contained in an exceedingly fine membrane, so that if it is sliced across with a knife, its contents will flow out. He also set right the errors of Fabricius, Parisanus and others, by showing that the chalazae were neither the seed of the cock nor the material out of which the embryo was formed, and, most important of all, by demonstrating that the cicatricula was the point of origin of the embryo. He denied, as against popular belief, that the hen contributed anything to the developing egg but heat, "For certain it is that the chicken is constituted by an internal principle in the egge, and that there is no accession to a complete and perfect egge by the Hennes incubation, but bare cherishing and protection; no more than the Hen contributeth to the chickens which are now hatched, which is only a friendly heat, and care, by which she defendeth them from the cold, and forreign injuries and helpeth them to their meat". Whether future work will still affirm that nothing is given to the egg by the hen except heat is beginning now to be in doubt, if the results of Chattock are correct.

In the description of the development of the embryo in the hen's egg, which remains to this day one of the most accurate, Harvey says with regard to the spot on the yolk, which had, of course, been seen and mentioned by many previous observers, "And yet I conceive that no man hitherto hath acknowledged that this Cicatricula was to be found in every egge nor that it was the first Principle of the Egge". His description of the beginning of the heart, that "capering bloody point" or "*punctum saliens*", is too famous to need more than a reference. He thought that the amniotic liquid was of "mighty use", "For while the embryos swim there, they are guarded and skreened from all concussion, contusion, and other outward injuries, and are also nourished by it".

Thus he made no advance on the opinion which had for long been held, namely, that the amniotic liquid or colliquamentum served for sustenance. "I believe", he says, "that this colliquamentum or water wherein the foetus swims doth serve for his sustenance and that the thinner and purer part of it, being imbibed by the umbilicall vessels, does constitute and supply the primo-genital parts, and the rest, like Milk, being by suction conveyed into the stomack and there concocted or chylified, and afterwards attracted by the orifices of the Meseraick Veins doth nourish and enlarge the tender embryo." His arguments for this are, (1) that swallowing movements take place, and (2) that the gut of the chicken is "stuft" with excrement which could hardly arise from any other source. He was thus led to divide the amniotic liquid into two quite imaginary constituents, a purer and "sincerer" part, which could be absorbed straight into the blood without chylification, and a creamless milky part which could not be treated so simply.

"About the fourth day", says Harvey, "the egg beginneth to step from the life of a plant to that of an animall." "From that to the tenth it enjoys a sensitive and moving soul as Animals do, and after that, it is compleated by degrees and being adorned with Plumes, Bill, Clawes and other furniture, it hastens to get out." These and other passages which deal with the forerunner of the theory of recapitulation are interesting, but we have already met essentially the same idea in Aristotle. Harvey contributed nothing new to it. The first point on which he went definitely wrong was the statement that he made that the heart does not pulsate before the appearance of the blood. No doubt his lack of microscopical facilities or of the desire to use them affords the reason for this error, but it was a very unfortunate one, for it was to a large extent upon it that he formulated his doctrine "the life is in the blood". For example, he says, "I am fully satisfied that the Blood hath a being before any other part of the body besides, and is the elder brother to all other parts of the foetus".

The yolk, Harvey thought, supplied the place of milk, "and is that which is last consumed, for the remainder of it (after the chicken is hatched and walks abroad with the Henne) is yet contained in its belly". He thus ranged himself with Alcmaeon and Abderhalden. All his remarks about the relationships of yolk and white in nutrition are worth consideration; in noting, for instance, that the yolk is the last to be consumed, he comes very near to anticipating the knowledge of the succession of energy-sources which we now possess (see Section 7·7). "In that Physitians affirme, that the Yolke is the hotter part of the egge, and the most nourishing, I conceive that they understand it, in relation to us, as it is become our nourishment, not as it doth supply more congruous aliment to the chicken in the egge. And this appeares out of our history of the Fabrick of the chicken; which doth first prey upon and devoure the thinner part of the white, before the grosser; as it were a more proper diet, and did more easily submit to transmutation into the substance of the foetus. And therefore the yolke seems to be a remoter and more deferred entertainment than the white; for all the white is quite and clean spent, before any notable invasion is made upon the yolke." A comparison between these simple facts and our knowledge of embryonic nutrition is most interesting (see Section 6·9).

In connection with Minot's distinction of the periods of embryonic growth, it is curious that Harvey says, "And now the foetus moves and gently tumbles, and stretcheth out the neck though nothing of a brain be yet to be seen, but merely a bright water shut up in a small bladder. And now it is a perfect Magot, differing only from those kinde of wormes in this, that those when they have their freedom crawle up and down and search for their living abroad, but this worm constant to his station, and swimming in his own provision, draws it in by his Umbilicall Vessels".

Sometimes Harvey confesses himself puzzled by problems which could only be solved by chemical means, yet it does not occur to him that this is the case. For instance, he enquires why heat will develop a chick out of a good egg but will only make a bad one worse. "Give me leave to add something here", he writes, "which I have tried often; that I might the better discerne the scituation of the foetus and the liquors at the seventeenth day to the very exclusion. I have boiled an egge till it grew hard, and then pilling away the shell and freeing the scituation of the chicken, I found both the remaining parts of the white, and the two parts of the yolk of the same consistence, colour, tast, and other accidents, as any other stale egge, thus ordered, is. And upon this Experiment, I did much ponder whence it should come to passe that Improlifical eggs should, from the adventitious heat of a sitting Henne, putrifie and stink; and yet no such inconvenience befall the Prolifical. But both these liquors (though there be a Chicken in them too, and he with some pollution and excrement) should be found wholesome and incorrupt; for that if you eat them in the dark after they are boyled, you cannot distinguishe them from egges that are so prepared, which have never undergone the hen's incubation." Harvey was never afraid of trying such tests on himself; in another place, for example, he says, "Eggs after 2 or 3 days incubation, are even then sweeter relished than stale ones are, as if the cherishing warmth of the hen did refresh and restore them to their primitive excellence and integrity". "And the yolke (at 14 days) was as sweet and pleasant as that of a newlaid egge, when it is in like manner boyled to an induration." Another matter on which Harvey set Fabricius right was on the question whether at hatching the hen helps the chicken out or the chicken comes out by itself. The latter was the belief held by Harvey, who said of Fabricius' arguments on this point that they were "pleasant and elegant, but not well bottomed".

On the great question of preformation *v.* epigenesis, Harvey keenly argued in favour of the latter view. "There is no part of the future foetus actually in the egg, but yet all the parts of it are in it potentially....I have declared that one thing is made out of another two several wayes and that as well in artificial as natural productions, but especially in the generation of animals. The first is, when one thing is made out of another thing that is pre-existent, and thus a Bedstead is made out of Timber, and a Statue out of a Rock, where the whole matter of the future fabrick was existent and in being, before it was reduced into its subsequent shape, or any tittle of the designe begun. But the other way is when the matter is both made and receiveth its form at the same time....So likewise in the Generation of Animals, some are formed and transfigured out of matter already concocted and grown and all the parts are made and distinguished together *per metamorphosin*, by a metamorphosis, so that a complete animal is the result of that generation; but some again, having one part made before another, are afterwards nourished, augmented, and formed out of the same matter, that is, they have parts, whereof some are before, and some after, other, and at the same time, are both formed, and grow....These we say are made *per epigenesin*, by a post-generation, or after-production, that is to say, by degrees, part after part, and this is more properly called a Generation, than the former....The perfect animals, which have blood, are made by Epigenesis, or superaddition of parts, and do grow, and attain their just future or ἄκμη after they are born....An animal produced by Epigenesis, attracts, prepares, concocts, and applies, the Matter at the same time, and is at the same time formed, and augmented....Wherefore Fabricius did erroniously seek after the Matter of the chicken (as it were some distinct part of the egg which went to the imbodying of the chicken) as though the generation of the chicken were effected by a Metamorphosis, or transfiguration of some collected lump or mass, and that all the parts of the body, at least the Principall parts, were wrought off at a heat or (as himselfe speaks) did arise and were corporated out of the same Matter." Nothing could be more plain than Harvey's teaching on epigenesis, so that he has precedence over Caspar Wolff on this matter.

On the relation between growth and differentiation Harvey has some valuable things to say. The term "nutrition" he restricted to that which replaces existent structures, and the term "augmentation" or "increment" to that which contributes something new. That process which led to greater diversity of form and complexity of shape he called "formation" or "framing". "For though the head of the Chicken, and the rest of its Trunck or Corporature (being first of a similar constitution) do resemble a Mucus or soft glewey substance; out of which afterwards all the parts are framed in their order; yet by the same Operatour they are together made and augmented, and as the substance resembling glew doth grow, so are the parts distinguished. Namely they are generated, altered, and formed at once, they are at once similar and dissimilar, and from a small similar is a great organ made." Harvey was thus very certain that the processes of growth in size and differentiation in shape went on quite concurrently, though he had no inkling of changes in the relative rapidity of each process. On this point he goes further than Fabricius. Fabricius thought that growth was a more or less mechanical process, taking its origin from the properties of elementary substances, but that differentiation was brought about by some more spiritual or subtle activity. "Fabricius", says Harvey, "affirmes amisse, that the Immutative Faculty doth operate by the qualities of the elements, namely, Heat, Cold, Moisture, and Dryness (as being its instruments) but the Formative works without them and after a more divine manner; as if (forsooth) she did finish her task with Meditation, Choice, and Providence. For had he looked deeper into the thing, he would have seen that the Formative as well as the Alterative Faculty makes use of Hot, Cold, Moist, and Dry, (as her instruments) and would have deprehended as much divinity and skill in Nutrition and Immutation as in the operations of the Formative Faculty her self." "I say the Concocting and Immutative, the Nutritive and Augmenting Faculties (which Fabricius would have to busie themselves only about Hot, Cold, Moist, and Dry, without all knowledge) do operate with as much artifice, and as much to a designed end, as the Formative faculty, which he affirms to possess the knowledge and fore-sight of the future action and use of every particular part and organ." Thus although in nearly every respect Harvey makes an advance on Fabricius, yet here he is retrograde, for, in the former's thought, the growth process at least had struggled towards a deterministic schema; with Harvey this movement is rigidly suppressed. "All things are full of deity" (*Jovis omnia plena*), said he, "so also in the little edifice of a chicken, and all its actions and operations, *Digitus Dei*, the Finger of God, or the God of Nature, doth reveal himself."

There can be no doubt that Harvey's leanings were vitalistic. In the following passage, he argues against both those who wished to deduce generation from properties of bodies (like Sir Kenelm Digby) and the Atomists; in other words, against the outlook of those types of mind which in later times were to build up biophysics and biochemistry. Aubrey notes that Harvey was "disdainfull of the chymists and undervalued them".

"It is the usual error of philosophers of these times", says he, "to seek the diversity of the causes of parts out of the diversity of the matter from whence they should be framed. So Physicians affirm, that the different parts of the body are fashioned and nourished by the different materials of blood or seed; namely the softer parts, as the flesh, out of a thinner matter, and the more earthy parts as the bones, out of grosser and harder. But this error now too much received, we have confuted in another place. Nor are they lesse deceived who make all things out of Atomes, as Democritus, or out of the elements, as Empedocles. As if (forsooth) Generation were nothing in the world, but a meer separation, or Collection, or Order of things. I do not indeed deny that to the Production of one thing out of another, these forementioned things are requisite, but Generation her self is a thing quite distinct from them all. (I finde Aristotle in this opinion) and I my self intend to clear it anon, that out of the same White of the Egge (which all men confesse to be a similar body, and without diversity of parts) all and every the parts of the chicken whether they be Bones, Clawes, Feathers, Flesh, or what ever else, are procreated and fed. Besides, they that argue thus assigning only a material cause, deducing the causes of Natural things from an involuntary or casual concurrence of the Elements, or from the several disposition or contriving of Atomes; they doe not reach that which is chiefly concerned in the operations of nature, and in the Generation and Nutrition of animals, namely the Divine Agent, and God of Nature, whose operations are guided with the highest Artifice, Providence, and Wisdome, and doe all tend to some certaine end, and are all produced, for some certaine good. But these men derogate from the Honour of the Divine Architect, who hath made the Shell of the Egge with as much skill for the egge's defence as any other particle, disposing the whole out of the same matter and by one and the same formative faculty." But although these are Harvey's theories, it is significant that in his preface he says, "Every inquisition is to be derived from its causes, and chiefly from the material and efficient", thus expressly excluding formal and final considerations. Certainly, as far as his practical work went, he was unaffected by them, and in the case of the egg-shell, for example, Harvey was not the man to say, "it is present for the protection of the embryo", and then to do or say nothing more. Such an explanation, though he might gladly accept it, was no bar to further exploration both by way of experiment and observation.

Harvey not only follows Aristotle in his good discoveries and true statements about the egg, but also, unfortunately, in his less useful parts, as, for example, when he devotes several pages to the discussion of how far the egg itself is alive, and whether there is any soul in subventaneous or unfruitful eggs. He decides that there is only a vegetative soul. On the other hand, he admirably refutes the opinion of those physicians—who were not few in number—who declared that the foetal organs were all functionless during foetal life. "But while they contende", he says, "that the mother's Blood is the nutriment of the foetus in the womb, especially of the *Partes Sanguineae*, the bloody parts (as they call them) and that the Foetus is at first, as if it were a part of the mother, sustained by her blood and quickened by her spirits, in so much that the heart beats not and the liver sanguifies not, nor any part of the Foetus doth execute any publick function, but all of them make Holy-Day and lie idle; in this Experience itself confutes them. For the chicken in the egge enjoyes his own Blood, which is bred of the liquors contained within the egge, and his Heart hath its motion from the very beginning, and he borroweth nothing, either blood or spirits, from the Hen, towards the constitution either of the sanguineous parts or plumes, as those that strictly observe it may plainly perceive." We have already seen how the Stoics in antiquity believed that the embryo was a part of the mother until it was born; from this idea the transition would be easy to the belief that all the organs in the embryo were functionless and dependent on the activity of the corresponding ones in the maternal organism.

One of Harvey's most important services to thought lay in his abolishing for good the controversy which had gone on ever since the sixth century B.C. about which part of the egg was for nutrition and which for formation. He had the sense to see that the distinction was a useless and baseless one—"There is no distinct part (as we have often said) or disposed matter out of which the Foetus may be formed and fashioned....An egge is that thing, whose liquors do serve both for the Matter and the Nourishment of the foetus....Both liquors are the nourishment of the foetus."

As regards spontaneous generation, Harvey considered that even the most imperfect and lowest animals came out of eggs. "We shall show", he writes, "that many Animals themselves, especially insects do germinate and spring from seeds and principles not to be discerned even by the eye, by reason of their contract invisible dimensions (like those Atomes, that fly in the aire) which are scattered and dispersed up and down by the winds; all which are esteemed to be Spontaneous issues, or born of Putrefaction, because their seed is not anywhere seen." Unfortunately, he never returned to this subject, for, as he himself informs us in another place, all the papers and notes in his house in London were destroyed at the time of the Civil War, so that what he had written on the generation of insects irretrievably perished.

Another point on which Fabricius had been in error was the appearance of bone and cartilage in the embryo. According to him, "Nature first stretcheth out the Chine Bone, with the ribbes drawn round it, as the Keel, and congruous principle, whereon she foundeth and finisheth the whole pile". This armchair conceit Harvey was easily able to destroy by a mere appeal to experience, but by experience also he came upon a fact less easily to be explained, namely, that the motion of the foetus began when as yet there was hardly any nervous system. "Nor is it less new and unheard of, that there should be sense and motion in the foetus, before his brain is made; for the Foetus moves, contracts, and extends himself, when there is nothing yet appears for a braine, but clear water." On the basis of this paradox Harvey may be said to be the discoverer of myogenic contraction, but he already could claim that distinction, for the first heart-beats are accomplished long before there are any nerves to the heart, as he himself points out. "We may conclude from this fact", he remarks, "that the heart and not the brain is the first principle of embryonic life", and he gives instances of physiological actions not under the conscious control of the individual, such as the reflexes, as we should call them, of the intestinal tract, and the emetic action of infusion of antimony which cannot be tasted much and "yet there passeth a censure upon it by the Stomack" and a vomit ensues. Thus, twenty-five years before Francis Glisson, Harvey had formulated, from embryological studies, the view that irritability was an intrinsic property of living tissues.

Both Harvey and Fabricius were very puzzled about the first origin of the blood. "What artificer", says Harvey, "can transform the two liquors into blood, when there is yet no liver in being?" It was to be a long time before this question was answered by Wolff's discovery of the blood islands in the blastoderm, and, even now, the chemistry of the appearance of haemoglobin is one of the most obscure corners of chemical embryology. The older observers explained it by considering the yolk to be akin to blood and ready to turn into it at the slightest inducement.

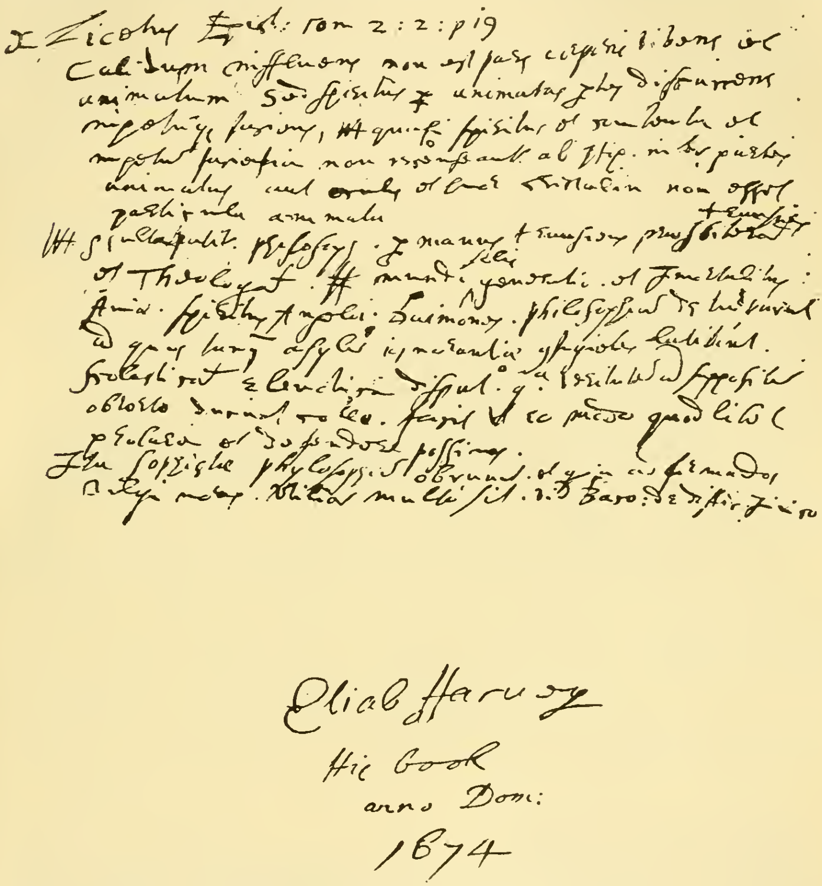
Another problem which neither Fabricius nor Harvey did anything to solve was the nature of the air-space at the blunt end of the egg. "Fabricius recounts several conveniences arising from it, according to its several magnitudes, which I shall declare in short, saying, It contains aire in it, and is therefore commodious to the Ventilation of the egge, to the Respiration, Transpiration, and Refrigeration, and, lastly, to the Vociferation of the Chicken. Whereupon, that cavity is at the first very little, afterwards greater, and at last greatest of all, according as the several recited uses do require."

As regards the placenta, Harvey took the side of Arantius and denied any connection between the maternal and foetal circulations. "The extremities of the umbilicall vessels", he said, "are no way conjoined to the extremities of the Uterine vessels by an Anastomosis, nor do extract blood from them, but are terminated in that white mucilaginous matter, and are quite obliterated in it, attracting nourishment from it." "Wherefore these caruncles may be justly stiled the Uterine Cakes or Dugs, that is to say, convenient and proportionate organs or instruments designed for the concocting of that Albuginous Aliment and for preparing it for the attraction of the veins." From this it would appear that Harvey regarded the uterine milk as the special secretion of the placenta, conveyed to the foetus through the umbilical cord. The nature of the uterine milk is still very imperfectly understood (see Section 21). Its discovery is usually attributed to Walter Needham, but various remarks in this chapter (Ex. LXX) seem to show that Harvey was well acquainted with it. In later times, it was regarded by some (Bohnius and Charleton in 1686, Zacchias in 1688 and Franc in 1722) as the sole source of foetal nourishment. Mercklin spoke of it in 1679 as "*materia albuginea, ovique albo non absimili*". Harvey often calls the placenta the uterine liver, no doubt only for this reason, but the remarkable appropriateness of the term was to become apparent in Claude Bernard's day. As regards the matter of the continuity of the maternal and foetal circulations, he criticises van Spieghel. "There came forth a book of late", he says, "wrote by one Adrianus Spigelius, wherein he treateth concerning the use of the umbilicall arteries and doth demonstrate by powerfull arguments that the Foetus doth not receive its Vital Spirits by the arteries from the Mother, and hath fully answered those arguments which are alledged to the contrary. But he might also as well have proved by the same arguments that the blood neither is transported into the Foetus from the mother's veines by the propagations of the umbilicall veins which is made chiefly manifest by the examples drawn from the Hen-Egge and the Caesarean Birth."

The least satisfactory parts of Harvey's book are the Exercitations LXXI and LXXII on the innate heat and the primigenial moisture. Here he becomes very wordy and highly speculative, and gives us little but a mass of groundless arguments. He devotes many pages to proving that the innate heat is the blood and to drawing distinctions between blood and gore, the one in the body, the other shed. In one place he speaks of the processes of generation as so divine and admirable as to be "beyond the comprehension and grasp of our thoughts or understanding". Two centuries previously Frascatorius had said precisely the same thing about the motion of the heart, and it was ironical that the very man who let the light in on cardiac physiology should in his turn despair of the future of our knowledge of embryonic development.

Harvey did not say much about foetal respiration, and his few remarks are contained in one of the "additional discourses". He is puzzled exceedingly by the question. But he comes very near indeed to the truth when he says, "Whosoever doth carefully consider these things and look narrowly into the nature of aire, will (I suppose) easily grant, that the Aire is allowed to animals, neither for refrigeration, nor nutrition sake. For it is a tryed thing, that the Foetus is sooner suffocated after he hath enjoyed the Aire, than when he was quite excluded from it, as if the heat within him, were rather inflamed than quenched by the aire". Had Harvey pursued this line of thought, and looked still more narrowly into the nature of air, he might have anticipated Mayow. He does say that he proposes to treat of the subject again, but he never did.

The mainspring of Harvey's researches on the does and hinds can be realised by a reference to Rueff's figures in Fig. 6. According to the Aristotelian theory, the uterus after fertile copulation would be full of blood and semen; according to the Epicurean theory (held by the "physitians") it would be full of the mixed semina. If this coagulated mass exists, said Harvey, it ought to be possible to find it by dissection, and this was what he tried to do. It soon became plain, as may be read in Ex. LXVIII, not only to Harvey but to the King and the King's gamekeepers, that no such coagulum existed,



Eliab Harvey
His Book
anno Dom:
1674

Fig. 7. Manuscript notes of Dr William Harvey.

and the result was made still more certain by means of segregation experiments which the King carried out at Hampton Court. Accordingly there was nothing to be done but to abandon all the older theories completely, and have recourse to some sort of hypothesis in which an *aura seminalis*, an "incorporeal agent" or a "kinde of contagious property" should bring about fertilisation. This was a perfectly sound deduction from Harvey's experiments, and did not then appear anything like so unsatisfactory as it does now, for Gilbert of Colchester was not long dead, the "lodestone" was beginning to be investigated by the virtuosi, and even such extravagances as Sir Gilbert Talbot's Powder "for the sympatheticall cure of wounds" were only with difficulty distinguishable from the real effects of magnetic force. Harvey's idea of fertilisation by contagion has recently been in a sense revived by the work of Shearer (see Section 4·2).

But to Harvey himself the subject of the action of the seed was hid in deep night, and he confessed that, when he came to it, he was "at a stand". Some very interesting light is thrown upon his mind in this connection by a copy of the *De Generatione Animalium* annotated by himself, and now in the possession of Dr Pybus, by whose courtesy and by that of Dr Singer, who has transcribed the notes, I have been enabled to study it. It was given by Harvey to his brother Eliab, whose name it still bears. The notes, which are on the fly-leaves, are written in much the same way as those famous ones which Harvey used for his lectures at the College of Physicians in London, and which have been reproduced in facsimile. There is the same mixture of Latin and English, and the same signs, such as WH, to denote thoughts claimed as original. A page is reproduced in Fig. 7.

For the most part, the notes are uninteresting and nothing but a confusion of Aristotelian terms. But one page is concerned with the mode of action of the seed, and here we can, as it were, see Harvey's mind wrestling with this most difficult of problems. He sees that odour and the sense of smell may give a clue. That his thoughts on this point were doomed to frustration as soon as eggs and spermatozoa were discovered does not detract from the interest of the struggle.

Quod facit semen fecundum

What makes the seed fertile is on the analogy of an injection. In fact, the injection causes disease in many cases, and that from a distance, both by another...and by the same... △ Venereal (?) disease corrupts coitus with a woman in whose uterus is the poison.

They do not [or do not yet ?] come forth in actuality but lie dormant as in fuel [? fomite]. Again, rabies in dogs lies dormant for many days on my own observation WH. Again, smallpox for days. Again, the generative seed, just as it (passes) from the male, lies dormant in the woman as in fuel (?).

Or else like a..., like light in stone..., the pupil in the eye, in sense motion,...in the body.

Like ferment, vapour, odour, rottenness...by rule.

Or like the smell given off by flowers.

Like heat, inflammation (?) △ in chalk (heat ?) both the wet form.... Like what is first...in the art of cooking...principles of vegetation and propagation. △ Dormice by hibernating...cleansing by water and all kinds of lotions, again for insects, as for their seeds as well (?). Or when a soul is a god present in nature, that is divine which it brings about without an organic body by means of law.

See Aristotle on sense and the objects of sense.

Marvels concerning odours and smells given off. Whether everything that can be smelt gives off something and so disperses (?) what is not without heat, or by destroying... attracts to itself.

△ Amongst inflammable (objects are) fire, naphtha, paper....

△ WH manus et odore car...anatomia manair....

△ Anat...post 4^{or} poras. otium inclinente die rursus quod prius et olefrere vid....Galen....

△ Mr. Boys spainel in Paris lay all ye third night and morning in getting dogg. Whelping dogg's sent (scent) are a stronger sent, vesting in vestigio alios ord...gr...lepris odore lepris esse libidine esse. Hors, the mare, *hors*, the cow, a bull per mutta millsa.

△ ...si lepra fracedo in farioli fader cupidinitus. Dogg ye otter in aquas fracedo vasorum ex sulpore?

Just as Aristotle put much of his best embryological work into his *Historia Animalium* and not into the work with the appropriate title, so Harvey has some admirable observations on the embryonic heart scattered through his *De Motu Cordis et Sanguinis in Animalibus*. Turning now to consider Harvey's influence on embryology, we must admit that it was in certain respects reactionary.

1. He did not break with Aristotelianism, as a few of his predecessors had already done, but on the contrary lent his authority to a moribund outlook which involved the laborious treatment of unprofitable questions.

2. His opposition to atomism and to "chymistry" precluded any close co-operation between his followers and those of the Descartes-Gassendi tradition.

3. Fabricius had elaborated a vitalistic theory of differentiation, but had allowed growth to be "natural" or mechanical. Harvey, however, made both growth and differentiation the results of an immanent spirit, a sort of divine legate.

But these failings are far outweighed by his positive services. It must always be remembered that he had no compound microscope, and had to rely, like Riolanus, on "perspectives", or simple lenses of very low power.

1. There can be no doubt that the doctrine *omne vivum ex ovo* was a tremendous advance on all preceding thought. Harvey's scepticism about spontaneous generation antedated by less than a century the experiments of Redi. It is important to note that he was led to his idea of the mammalian ovum by observations on small conceptions surrounded by their chorion and no bigger than eggs, for the true ovum itself was not discovered until the time of de Graaf and Stensen.

2. He identified definitely and finally the cicatricula on the yolk-membrane as the spot from which the embryo originated.

3. He denied the possibility of generation from excrement and from mud, saying that even vermiparous animals had eggs.

4. He discussed the question of metamorphosis (preformation) and epigenesis, and decided plainly for the latter, at any rate for the sanguineous animals.

In addition to these achievements, there are others, perhaps less striking, but equally important.

5. He destroyed once and for all the Aristotelian (semen-blood) and Epicurean (semen-semen) theories of early embryogeny. This was perhaps the biggest crack he made in the Peripatetic teaching on development; but, in spite of it, Sennertus, van Linde and Sylvius adhered to the ancient views, and Cyprianus, in 1700, had the distinction of being the last to support them in a scientific discussion, though Sterne, as late as 1759, referred to them in a way that shows they still lived on in popular thought.

6. He handled the question of growth and differentiation better than any before, anticipating the ideas of the present century.

7. He settled for good the controversy which had lasted for 2200 years as to which part of the egg was nutritive and which was formative, by demonstrating the unreality of the distinction.

8. He set his predecessors right on a very large number of detailed points, such as the nature of the placenta.

9. He made a great step forward in his theory of foetal respiration, though here he did not consolidate the gain.

10. He affirmed that embryonic organs were active, and that the embryo did not depend on external aid for its principal physiological functions.

But all these titles to remembrance, great as they are, do not account for the peculiar fascination of Harvey. A little of it is perhaps due to his imaginative style, which comes out clearly in Martin Llewellyn's English version. A word of censure is due to Willis for transmuting it in his translation into the dull and pedestrian style of 1847. None who reads the 1653 edition of Harvey can ever forget such metaphors as this, "For the trunck of the body hitherto resembles a skiff without a deck, being in no way covered up by the anteriour parts"; or the vigour of diction which promotes such remarks as, "In a hen-egge after the tenth day, the heart admits no spectators without dissection"; or again, "For while the foetus is yet feeble, Nature hath provided it milder diet and solider meats for its stronger capacity, and when it is now hearty enough, and can away with courser cates, it is served with commons answerable to it. And hereupon I conceive that perfect eggs are not onely party-coloured, but also furnished with a double white"; or, lastly, "An egge is, as it were, an exposed womb; wherein there is a substance concluded, as the Representative and Substitute or Vicar of the breasts".

In this connection, it would be a pity not to quote from the verses which Llewellyn prefixed to his translation of Harvey's book. After describing the controversies that followed the *De Motu Cordis* he wrote

A Calmer Welcome this choice Peice befall,
Which from fresh Extract hath deduced all,
And for Belief, bids it no longer begg
That Castor once and Pollux were an Egge:
That both the Hen and Houswife are so matcht,
That her Son born, is only her Son hatcht;
That when her Teeming hopes have prosp'rous bin,
Yet to conceive, is but to lay, within.
Experiment, and Truth both take thy part:
If thou canst 'scape the Women! there's the Art.

Live Modern Wonder, and be read alone,
Thy Brain hath Issue, though thy Loins have none.
Let fraile Succession be the Vulgar Care;
Great Generation's Selfe is now thy Heire.

Curiously enough, the "calmer welcome" which Martin Llewellyn hoped for actually happened. Harvey's book was so well reasoned and based on such good observations that it produced only two answers, and they were of little importance. Janus Orcham took exception to Harvey's finding no seed in the uterus and suggested that it had vaporised like a steam, but his Aristotelian leanings were promptly detected and castigated by Rallius. Matthew Slade, taking the pseudonym of Theodore Aldes, published in 1667 his *Dissertatio epistolica contra D. G. Harveium*, which was, in his own words, "a detection of one or two errors in that golden book on the generation of animals of William Harvey, greatest of physicians and anatomists". The errors were purely anatomical, and ab Angelis defended Harvey against Slade's attack, claiming that the "errors" were not errors at all. A manuscript work of Slade's appears to be extant.

Harvey's influence was evidently speedily felt by his contemporaries. Strauss soon wrote a rather poor book on the bird's egg in imitation of him. But the best instance is that in 1655, very soon after the publication of Harvey's book, William Langly, "an eminent senator and physician of Dordrecht", made a great many experiments on the development of the hen's egg. Buffon says that he worked in 1635, i.e. before Harvey, but this is not the case, for in his observations which were published by Julius Schrader in 1674 the later date is given several times. Langly mentions Harvey more than once, and evidently followed his example in careful observation, for his text is concise and accurate and his drawings very noteworthy.

Julius Schrader included Langly's work in a composite volume containing a well-arranged epitome of Harvey's book on generation and some observations of his own on the hen's egg. The book was dedicated to Matthew Slade and J. Swammerdam. On the practical side Schrader added nothing memorable to Harvey and Langly, but it is noteworthy that the mammalian embryo was throughout these centuries more popular material than that of the chick. Out of fifty embryologists between Harvey and Haller, the names of Langly, Schrader, Malpighi and Maître-Jan practically exhaust the list of those who studied the egg of the hen. This rather unfortunate orientation of mind doubtless sprang from the strong influence of medicine, and especially obstetrics, on seventeenth and eighteenth century embryology.

3·5. Gassendi and Descartes: Atomistic Embryology

Harvey's death took place in 1657. The following year saw the publication of Pierre Gassendi's *Opera Omnia*, and thus brought in an entirely new phase in embryology. Together with Réné Descartes' treatise on the formation of the foetus, Gassendi's *De generatione animalium et de animatione foetus* marks a quite different attitude to the subject. Harvey had adopted a rather contemptuous position about the "corpuscularian or mechanical philosophy", which was then coming in, and had expected even less help from it in the solution of his problems than from his equally despised "chymists". Gassendi now set out to show that the formation of the foetus could be explained on an atomistic basis: and, using the Galenic physiology and the new anatomy as a framework, he set forth his theory in full. As we read it through at the present day, however, we cannot avoid the confession that it was not a success. In spite of his frequent quotations from Lucretius and his persuasive style, it does not carry conviction. The truth of the matter was that the time was not ripe for so great a simplification. The facts were insufficiently known, and that Gassendi is not quite as interested in them as he is in his theory is shown by the circumstance that he only mentions Harvey once.

Gassendi examines in turn the Aristotelian and the Epicurean doctrines of embryogeny and rejects them both, the former on the ground that the change from egg to hen is too great and difficult for anything so shadowy and ghost-like as a "form" to accomplish, and the latter because it leaves no room for teleology. He therefore adopts as the basis of his system atomism + preformationism, alleging that all the germs of living things were made at the creation, but that they come to their perfection as atomic congregations in an atomistic universe. Thomas' monograph is a valuable help to the study of this very interesting thinker.

At exactly the same time, Descartes was speculating on the same subject. Added to his posthumous *De Homine Liber* (1662) is a treatise on the formation of the foetus. He may also have written a work *On the generation of animals*, for a manuscript with that title was found among his papers after his death, and was believed to be in his handwriting. There is evidence, however, that it is not his, and though it was published in Cousin's edition of his works, we may safely neglect it, agreeing, in the words of that editor, that it is "a fragment in which very mediocre and often quite false ideas struggle to light through the medium of a style devoid alike of clarity and of grandeur". It must be admitted, however, that even his main treatise is very confused. It suffers from containing in its earlier part a great deal of matter which really belongs to the physiological text-book which immediately preceded it. Thus it begins abruptly in the middle of a disquisition on the error of attributing bodily functions to the soul. Before long, however, it warms to its theme, and a conception of growth is outlined. "When one is young, the movement of the little threads which compose the body is less slow than it is in old age, because the threads are not so tightly joined one to the other, and the streams in which the solid particles run are large, so that the threads become attached to more matter at their roots than detaches itself from their extremities, so that they grow longer and thicker, in this way producing growth." The fourth part of the book is called, strangely enough, a Digression, in which the formation of the animal is spoken of. The mixture of seeds is then described, and a theory of the formation of the heart is attempted by means of an analogy with fermentation. The explanation is unconvincing, but has a certain interest as showing chemical notions beginning to permeate biological thought. However, Descartes' way of looking at development was thoroughly novel, as is illustrated by the following citation. "How the heart begins to move....Then, because the little parts thus dilated, tend to continue their movement in a straight line, and because the heart now formed resists them, they move away from it and take their course towards the place where afterwards the base of the brain will be formed, they enter into the place of those that were there before, which for their part move in a circular manner to the heart and there, after waiting for a moment to assemble themselves, they dilate and follow the same road as the aforementioned ones, etc." Descartes, in fact, with premature simplification, was trying to erect an embryology *more geometrico demonstrata.* That he failed in the attempt was as obvious to his contemporaries as it is to us—"We see", said Garden, "how wretchedly Descartes came off when he began to apply the laws of motion to the forming of an animal". In doing so, he was many years before his time; Borelli had done all that could be done at that period in that direction, and, significantly enough, he left embryology alone. The rest of Descartes' book is exactly like the citations which have been given, only applied to each organ and part in turn; he practically uses the traditional teaching as a scaffolding in which to interweave his mechanical theory, and he discovers no new facts.

But in the history of embryology these men and their writings have a very great significance. Impressed by the unity of the world of phenomena, they wished to derive embryology as well as physics from fundamental laws. This attempt, which resulted in a Galen-Epicurus synthesis on the one hand and a Galen-Descartes synthesis on the other, must be regarded as a noble failure. Its authors did not realise what a vast array of facts would have to be discovered before a mechanical theory could with any justice be applied to explain them. Gassendi and Descartes were like the Ionian nature-philosophers, propounding general laws before the particular instances were accurately known. Their ineffectiveness arises from the fact that they did not themselves appreciate this, and consequently worked out their idea in a prolix detail, the whole of which was inevitably doomed to the scrap-heap from the very beginning. But the spark was not to die; and if anywhere in this history we are to find the roots of physico-chemical embryology, we must pause to recognise them here.

Much less well known, but not without interest, was the *Dissertatio de vita foetus in utero* of Gregorius Nymmanus, which appeared in the same year as the second edition of Descartes' book, 1664. Nymmanus writes with a very beautiful Latin style, and expresses himself with great clearness. His proposition is, he says, "That the foetus in the uterus lives with a life of its own evincing its own vital actions, and if the mother dies, it not uncommonly survives for a certain period, so that it can sometimes be taken alive from the dead body of its mother". In supporting this thesis, Nymmanus answers the arguments of those who had held that the lungs and heart of the foetus were inactive *in utero*. Fabricius, Riolanus and Spigelius all proved, says Nymmanus, that the mother and the foetus by no means necessarily die at the same time. "The essential life", he says, "is the soul itself informing and activating the body, the accidental life is the acts of the soul which it performs in and with the body." Though the foetus cannot be said to have life in the latter sense, it can in the former. The foetus, says Nymmanus, prepares its own vital spirits and the instruments of its own soul; there is no nerve between it and its mother. If, he says, the foetal arteries got their sphygmic power from the maternal heart, they would stop pulsating when the umbilical cord was tied, but this is not the case. The pulse of the embryo is therefore due to the foetal heart itself. Galen, says Nymmanus, was aware of this, but did not understand the meaning of it. Again, the foetus *in utero* moves during the mother's sleep, and *vice versa*. Nymmanus' dissertation is an interesting study in the transition from theological to scientific embryology which took place all through the seventeenth century, and may be followed in the writings of Varandaeus, de Castro, Dolaeus, Hildanus, Scultetus, Ammanus, Augerius and Garmannus. The problem of animation-time, a more metaphysical aspect of the same question, was still being handled, but less attention was being paid to it than formerly. Honoratus Faber's *De Generatione Animalium* of 1666 does not belong to its period. Its author, a Jesuit, proceeds in scholastic fashion to lay down four definitions, three axioms, one hypothesis, and seventy-seven propositions, in the last of which he summarises his conclusions. He is interesting in that he displayed a disbelief in spontaneous generation, thereby anticipating Redi, and he is careful to mention the work of Harvey, but nevertheless his treatise is of little value. His chief importance is that he is an epigenesist, and therefore demonstrates to us how the true opinion was becoming accepted, when Malpighi's brilliant observations and bad theory sent it out of favour, and prepared the way for the numerous controversies of the following century.

3·6. Walter Needham and Robert Boyle

It was in 1666 also that the following appeared in the *Philosophical Transactions of the Royal Society*:

A way of preserving birds taken out of the egge, and other small faetus's:
communicated by Mr. Boyle.

When I was sollicitous to observe the Processe of Nature in the Formation of the Chick, I did open Hens Eggs, some at such a day, and some at other daies after the beginning of the Incubation, and carefully taking out the Embryo's, embalmed each of them in a distinct Glass (which is to be carefully stopt) in Spirit of Wine; Which I did, that so I might have them in readinesse to make on them, at any time, the Observations, I thought them capable of affording; and to let my Friends at other seasons of the year, see, both the differing appearances of the chick at the third, fourth, seventh, fourteenth, or other daies, after the eggs had been sate on, and (especially) some particulars not obvious in chickens, that go about, as the hanging of the Gutts out of the Abdomen, etc. How long the tender Embryo of the Chick soon after the Punctum saliens is discoverable, and whilst the bodie seems but a little organized Gelly, and some while after that, will be this way preserv'd, without being too much shrivel'd up, I was hindred by some mischances to satisfie myself; but when the Faetus's, I took out, were so perfectly formed as they were wont to be about the seventh day, and after, they so well retained their shape and bulk, as to make me not repent of my curiosity; And some of those, which I did very early this Spring, I can yet shew you.

Boyle said in conclusion that he sometimes also "added Sal Armoniack, abounding in a salt not sowre but urinous".

In the same year that Nymmanus' book appeared, Nicholas Stensen, that great anatomist, later a Bishop, who was also to all intents and purposes the founder of geology, published his *De musculis et glandulis specimen*, in which Coiter's observations on the vitelline duct and the general relations between embryo and yolk in the hen's egg were made again and confirmed. About this time also Deusingius described his case of abdominal pregnancy, and was thus the first anatomist to draw attention to this phenomenon.

In 1667 Stensen published his *Elementorum myologiae specimen*, in which he described the female genital organs of dogfishes. He demonstrated eggs in them and affirmed that the "testis" of women ought to be regarded as exactly the same organ as the "ovary" or "roe" of ovipara. At the time he carried the suggestion no further, but it was an extremely fruitful one, and it is surprising that it did not create more interest, for it was exactly what Harvey had been looking for. Nothing obvious having been found in the uteri of King Charles' does, and the conviction yet being very strong that viviparous conceptions really came from eggs, Stensen's minute ova supplied the fitting answer to the question. Thus Harvey and Stensen between them substituted the modern knowledge of mammalian ova for the ancient theory of the coagulum all in the space of fourteen years. The other event for which the year 1667 is remarkable is the *De Formato Foetu* of Walter Needham. Needham was a Cambridge physician who went to Oxford to study in the active school of physiological research which such men as Christopher Wren, Richard Lower, John Ward and Thomas Willis were making famous. His book on the formation of the embryo, written later (and dedicated to Robert Boyle), after he had been in practice in Shropshire for some time, is important because it is the first book in which definite chemical experiments on the developing embryo are reported, and also because it contains the first practical instructions for dissections of embryos.

Sir Thomas Browne had, as we have already seen, made experiments of a chemical nature on the constituents of birds' eggs and of the eggs of amphibia, but he did not analyse them after any development had been allowed to take place. He may therefore be regarded as the father of the static aspect of physico-chemical embryology, while Walter Needham may be regarded as the founder of the dynamic aspect. The practical difficulties of these pioneers of animal chemistry may be seen in such a book of practical instructions as Salmon's *General Practise of Chymistry* of 1678. They had no satisfactory glassware, no pure reagents, the methods of heating were incredibly clumsy, and there was no means of measuring either heat or atmospheric pressure.

In the review of Needham's book which is to be found in the *Philosophical Transactions of the Royal Society* for September 1667 there occurs the sentence, "These humors (the amniotic, allantoic, etc.) he saith, he hath examined, by concreting, distilling, and coagulating them; where he furnishes the Reader with no vulgar observations". What were these observations? They are to be found in the chapter entitled "The nature of the humours":

"I now proceed to speak of this other nutritive liquor round about the urine itself which latter is plainly separated by the kidneys and the bladder. These liquors also proceed from the blood and seem similar to its serum but yet they are different from it. For when fire is applied to them in an evaporating basin [*cochlea*] they do not coagulate, as the blood-serum always does. Indeed, not even the colliquamentous liquid of the egg itself coagulates in this manner, although it is formed from juices which are evidently liable to coagulation—in the same way humours differ from themselves before and after digestion, filtration, and the other operations [*mangonia*] of nature. All, when distilled, give over a soft and clear water [*mollem et lenem*] very like distilled milk. This property is common to the liquor of the allantoic space, along with the rest. Because when the salts are not yet made wild and exalted the serum of the blood remains still quite soft and does not give proof of a tartaric or saline nature. Indeed, the first urine of an infant is observed by nurses to be not at all salt, but in older animals, when I distilled it in an alembic, I seemed to observe a little volatile salt at the small end [*in capitello*]. Coagulations attempted by acids happened differently in respect of the different humours. For when I poured a decoction of alumina into the liquor of the cow's amnios it exhibited a few rather fine coagulations but they were clearly white. The allantoic juice, however, was precipitated like urine. Spirits of vitriol and vinegar brought about less results than alumina in each case. Spontaneous concretions I found also in the later months; these I discovered in both places. They are more frequent and larger, however, within the allantoic membrane."

From the above excerpt, which contains the account of all that Needham did on the chemical composition of the embryonic liquids, it can be seen that he treated the whole matter more dynamically than Browne. He was the first to describe the solid bodies in the amniotic fluid (see Jenkinson) and his chemical experimentation was all pioneer work.

His book has other merits, however. In the first chapter, he refutes the theory which Everard had propounded, that the uterine milk was identical with the contents of the thoracic duct, conveyed by lymphatic vessels to the uterus from the lacteals of Aselli, instead of elsewhere, and he shows that arteries must be the vessels bringing the material to the womb. The second chapter deals with the placenta "where he giveth a particular account of the double Placenta or Cake, to be found in Rabbets, Hares, Mice, Moles, etc., and examines the learned Dr Wharton's doctrine, assigning a double placenta to at least all the viviparous animals, so as one half of it belongs to the Uterus, the other to the Chorion, shewing how far this is true, and declaring the variety of these Phaenomena. Where do occur many uncommon observations concerning the difference of Milk [uterine] in ruminating and other animals, the various degrees of thickness of the uterin liquor in oviparous and viviparous creatures". He describes the human placenta very correctly indeed. "The use of the placenta is known to be to serve for conveighing the aliment to the foetus. The difficulty is only about the manner. Here are examined three opinions, of Curvey, Everhard, and Harvey. The two former do hold that the foetus is nourished only from the Amnion by the mouth; yet with this difference, that Curvey will have it fed by the mouth when it is perfect, but whilst it is yet imperfect, by filtration through all the pores of the body, and by a kind of juxtaposition: but Everhard, supposing a simultaneous formation of all the instruments of nutrition together and at first, and esteeming the mass of bloud by reason of its asperity and eagerness unfit for nutrition, and rather apt to prey upon than feed the parts, maintains, that the liquor is sucked out of the amnion by the mouth, concocted in the stomack, and thence passed into the Milky Vessels even from the beginning. Meantime they both agree in this, that the embryo doth breath but not feed through the umbilicall vessels. This our Author undertakes to disprove; and having asserted the mildness of, at least, many parts of the bloud, and consequently their fitness for nutrition, he defends the Harveyan doctrine of the colliquation of the nourishing juyce by the Arteries and its conveyance to the foetus by the veins."

In the third chapter Needham gives the first really comparative account of the secondary apparatus of generation, enunciating the rather obvious rule that in any given case the number of membranes exceeds the number of separate humours by one. He affirms that all the humours are nutritive save the allantoic. It had previously been held that all fish eggs were of one humour only, but he points out that a selachian egg has its white and yolk separate. He gives the results of his chemical experiments at this point, and suggests that the noises heard from embryos *in utero* and *in ovo* may be due to the presence of air or gas in the amniotic cavity, thus forming a link between Leonardo and Mazin. In his fourth chapter he deals with the umbilical vessels and the urachus, and here he claims priority over Stensen for the discovery of the *ductus intestinalis* in the chick, referring to Robert Boyle, Robert Willis, Richard Lower and Thomas Millington, to whom, he says, he showed the duct before Stensen published his observations on it. The fifth chapter is concerned with the *foramen ovale*, and the arterial and venous canals, and with the foetal circulation in general. The sixth is about respiration or "biolychnium", and in it Needham writes against the conception of a vital flame, alleging cold-blooded animals, etc., in his favour, but here he takes a retrograde step, for he argues that the use of the lungs is not for respiration but to "comminute the bloud and so render it fit for a due circulation". "The seventh and last chapter contains a direction for the younger Anatomists, of what is to be observed in the dissection of divers animals with young, and first, of what is common to the viviparous, then, what is peculiar to severall of them, as, a sow, mare, cow, ewe, she-goat, doe, rabbet, bitch, and a woman, lastly, what is observable in an Egg, skate, salmon, frog, etc. All is illustrated with divers accurate schemes."

The subsequent course of chemical embryology in the seventeenth century may be put in a very few words. Marguerite du Tertre incorporated in her obstetrical text-book of 1677 the results of some similar experiments to those of Needham. "If you heat the (amniotic) liquor", she says, "it does not coagulate, and if you boil it it flies away leaving a crass salt like urine, but if you heat the serosity of blood, it solidifies as if it were glue." The same observation was recorded by Mauriceau in 1687, who concluded, with some common sense, that, as there was so little solid matter present, the liquid could not be very nutritive; and by Case in 1696, who said, "In this juice the plastic and vivifying force resides, for although to our eyes it looks in colour and consistency like the serum of the blood, yet it is absolutely [*toto coelo*] different; for if a little of the former is slowly evaporated [*si in cochleari super ignem detines*] no coagulation will ever appear." Lister said this once more in 1711, but with Boerhaave's work of 1732 the subject entered a new phase.

In 1670 Theodore Kerckring published an adequate work on foetal osteology, and, two years later, de Graaf and Swammerdam, making full use of the opportunities afforded them by the invention of the microscope, described in detail the ova of mammalia, thus demonstrating the truth of Stensen's suggestion of some years before. It is important to note that these workers mistook the "Graafian follicles" for the eggs—a mistake which was not rectified till the time of von Baer. Stensen himself published not long after an account of these eggs also, but he was by then too late to gain the priority of demonstration. Portal's claim that Ferrari da Grado, who lived in the fifteenth century, was the true discoverer of mammalian ova has been disproved by Ferrari; and, although it is true that Volcher Coiter described what we now call the Graafian follicles, he did not recognise in any way their true nature.

De Graaf's discovery was confirmed in 1678 by Caspar Bartholinus, and, in 1674, by Langly, whose original observations had been made, so it was said, in 1657, the year of Harvey's death. If this is true, Langly has the priority of observation, Stensen of theory and de Graaf of demonstration.

3·7. Marcello Malpighi: Micro-Iconography and Preformationism

In the year 1672, Marcello Malpighi, who had for many years previously been working on various embryological problems with the aid of the simple microscope, published his tractates *De Ovo Incubato* and *De Formatione Pulli in Ovo*. In spite of its great importance, there is not much to be said about it, for it is anything but a voluminous work. The plates in which Malpighi represented the appearances he had seen in his examination of the embryo at different stages are beautiful, and some of them are reproduced. Description of the embryo was now pushed back into the very first hours of incubation, and it is interesting to note that Malpighi could not have done his work without Harvey, whose name he mentions on his first page, and who pointed out the cicatricula as the place where development began, and therefore, as Malpighi must have reasoned, the place where microscopic study would be very profitable. Now for the first time the neural groove was described, the optic vesicles, the somites, and the earliest blood-vessels.

Malpighi opened the modern phase of the controversy preformation *versus* epigenesis by supporting the former view. Embryogeny, he held, is not comparable to the building of an artificial machine, in which one part is made after another part, and all the parts gradually "assembled", but takes place rather by an unfolding of what was already there, like a Japanese paper flower in water. He was led to this belief by the fact that development goes on after fertilisation as the egg passes down the oviduct, and in the most recently laid eggs gastrulation is already over, so that in his researches he could never find an absolutely undivided egg-cell. It is curious to note that he says his experiments were done "*mense Augusti, magno vigente calore*", so that more than a usual degree of development would have taken place overnight. Had he examined the cicatriculae in hens' eggs before laying, he would very probably not have formed this theory, and the epigenesis controversy would have been settled with Harvey. Another influence which was unfavourable to the epigenetic position was that it was Aristotelian, and therefore unfashionable. Yet Malpighi's view was much more sensible than many which succeeded it, for he did not maintain a perfectly equal swelling up of all parts existing at the start, but rather an unequal unfolding, a distribution of rate of growth at different times and in different regions of the body. Thus he says, "Now, as Tully says, Death truly belongs neither to the living nor to the dead, and I think that something similar holds of the first beginnings of animals, for when we enquire carefully into the production of animals out of their eggs, we always find the animal there, so that our labour is repaid and we see an emerging manifestation of parts successively, but never the first origin of any of them".

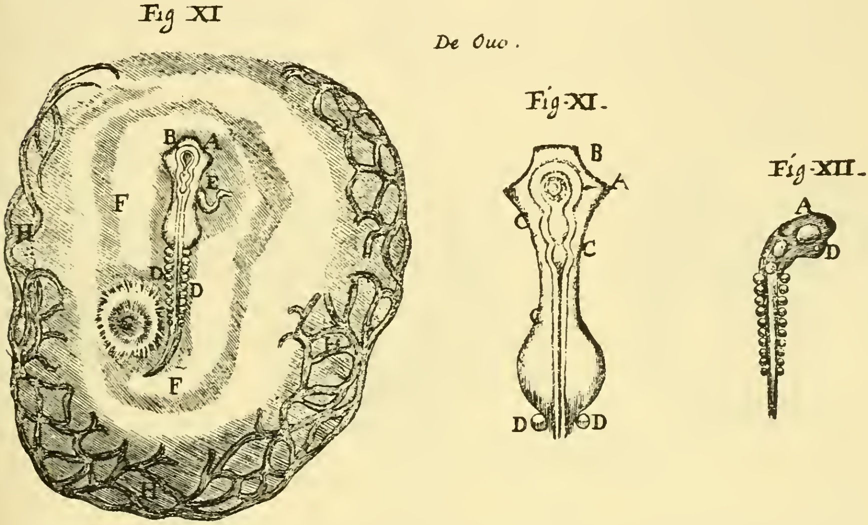


Fig. 8. Malpighi's drawings of the early stages of development in the chick embryo.

What had been an unfounded speculation for Seneca in antiquity and for Joseph de Aromatari and Everard in late times was now set upon an apparently firm experimental basis by Malpighi.

It is most instructive to note the difference in the attitudes of Langly and Schrader respectively towards the preformation question. Langly has no doubts about it, nor has Faber; they both follow Harvey and epigenesis unquestioningly, but Schrader, although he believes in epigenesis on the whole, is not at all certain about it. His friend, Matthew Slade, he says, brought the epistle of Joseph de Aromatari to his attention, and what with that and the unexplained observations of Malpighi on the pre-existence of the embryo, he is not willing to deny all value to preformationist doctrine. Others were bolder. It was immediately seized upon by Malebranche, the

Streeter of his age, who, in his *Recherche de la Vérité* of 1672, realised its philosophical possibilities, and gave it a kind of metaphysical sanction. That mystical microscopist, Swammerdam, made use of it as an explanation of the doctrine of original sin. In a remarkably short space of time it was a thoroughly established piece of biological theory.

Malebranche refers to it in his *Recherche de la Vérité* in the chapter where he treats of optical illusions and emphasises the deceitfulness and inadequacy of our senses. "We see", he says, "in the germ of a fresh egg which has not been incubated an entirely formed chicken. We see frogs in frogs' eggs and we shall see other animals in their germs also when we have sufficient skill and experience to discover them. We must suppose that all the bodies of men and animals which will be born until the consummation of time will have been direct products of the original creation, in other words, that the first females were created with all the subsequent individuals of their own species within them. We might push this thought further and belike with much reason and truth, but we not unreasonably fear a too premature penetration into the works of God. Our thoughts are, indeed, too gross and feeble to understand even the smallest of his creatures." Malebranche, who was a priest of the Oratory of the Cardinal de Bérulle, took an ardent interest in the scientific life of his time—for example, in a letter to Poisson, the Abbé Daniel wrote, "Reverend Father, M. Malebranche has written to me saying that he has installed an oven in which he has hatched eggs. He has already opened

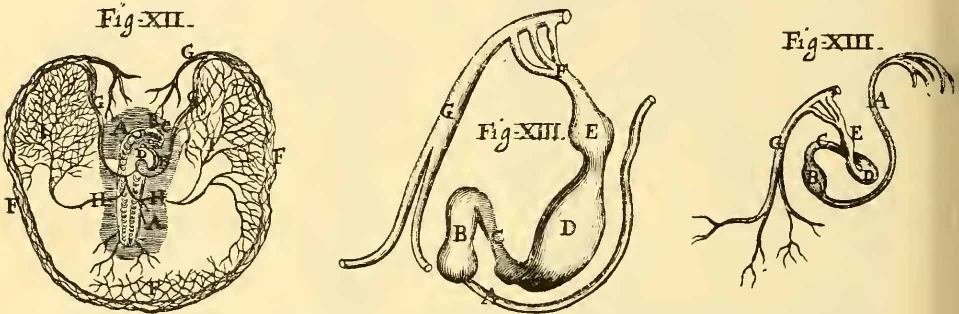


Fig. 9. Malpighi's drawings of the chick embryo's blood-vessels.

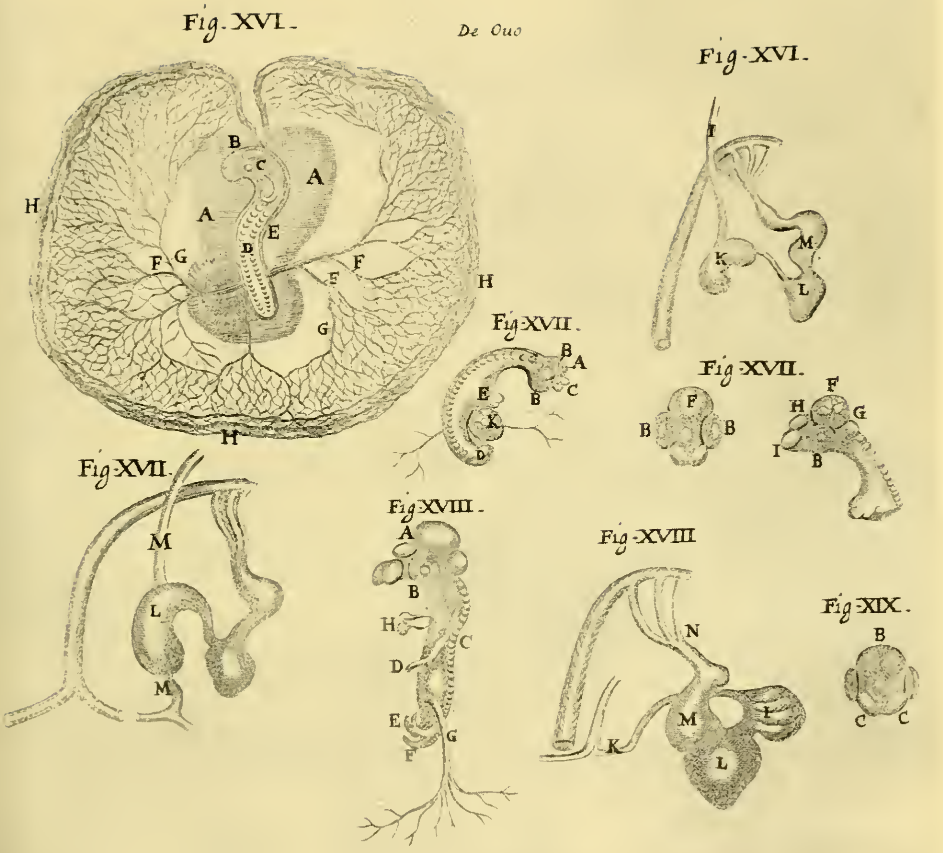


Fig. XVI.
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Fig. XIX.
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ILLUSTRATIONS FROM MALPIGHI'S *DE OVO INCUBATO* OF 1672

Showing the early stages of the development of the chick, somites, area vasculosa, etc.

some and has been able to see the heart formed in them and beating, together with some of the arteries" (Blampignon).

Swammerdam's support for preformation came from a different angle. He had been investigating insect metamorphosis, and, having hardened the chrysalis with alcohol, had seen the butterfly folded up and perfectly formed within the cocoon. He concluded that the butterfly had been hidden or masked (*larvatus*) in the caterpillar, and thence it was no great step to regard the egg in a similar light. Each butterfly in each cocoon must contain eggs within it which in their turn must contain butterflies which in their turn must contain eggs, and so on. Before long, Swammerdam extended this theory to man. "In nature", he said, "there is no generation but only propagation, the growth of parts. Thus original sin is explained, for all men were contained in the organs of Adam and of Eve. When their stock of eggs is finished, the human race will cease to be."

In 1684 Zypaeus reported that he had seen minute embryos in unfertilised eggs, and there were other similar claims. "*Hinc recentiores physiologi*", said Schurigius in 1732, "*hominem in ovulis delineatum quoad omnes partes in exiguis staminibus ante conceptionem existere statuunt.*"

Swammerdam cannot be regarded simply as one of the principal pillars of the preformation theory. His own embryological researches, which were made chiefly on the frog, were remarkable in many ways. He was the first to see and describe the cleavage of the egg-cell and later segmentation. He said that there was a time during the development of the tadpole when its body consisted of granules (*greynkens* or *klootkens*), but as these grew smaller and much more numerous they escaped his penetration. Leeuwenhoek also saw these cells, and his account was published long before Swammerdam's, but his observations on the rotating embryos of *Anodon* and the eggs of fleas were equally interesting.

3·8. Robert Boyle and John Mayow

In 1674 John Mayow, a young Oxford physician, published his tractate, *De Respiratione Foetus in Utero et Ovo*, which was included as one of the parts of his *Tractatus Quinque medico-physici* in that year. Mayow was the first worker to realise that gaseous oxygen, or, as he termed it, the "nitro-aerial" vapour, was the essential factor in the burning of a candle and the respiration of a living animal. His work was forgotten until Beddoes drew attention to it in 1790, but since then many have praised it and Schultze makes him the equal of Harvey.

The reason why he became interested in embryology is given in the opening sentences of his work. "Since the necessity of breathing", he says, "is so essential to the sustaining of life that to be deprived of air is the same as to be deprived of common light and vital spirit, it will not be out of place to enquire here how it happens that the foetus can live though imprisoned in the straits of the womb and completely destitute of air." He first of all gives an account of the opinions held about foetal respiration and the umbilical cord. He says that he disagrees (1) with the view that the embryo breathes *per os* while it is in the womb, for there is no air in the amnion and the *suctio infantuli* proves nothing; and (2) with the view propounded by Spigelius that the umbilical vessels existed to supply blood to the placenta for the nourishment of the latter. If this were the case, he says, the membranes in the hen's egg could not be formed before the vitelline vein, as they are, and in cases of foetal atrophy the placenta would always die and be corrupted too, which does not happen. Nor does he support the view of Harvey (3) that the umbilical vessels supply blood for the concoction and colliquation of the food of the foetus, for why should not the embryonic body prepare its own nutritious juice before birth just as it does afterwards. He further thinks the theory (4) that the umbilical vessels are for carrying off surplus foetal nourishment quite untenable and as little likely as the theory (5) that they exist for the object of allowing a foetal circulation—for this could just as well be accomplished through the vessels which exist in the embryonic body.

Mayow decides therefore for the opinion of *divino sene Hippocrate* and Everard that the umbilicus is a respiratory mechanism, carefully dissociating himself, however, from the hypothesis of Riolanus that the umbilical cord with all its windings is so arranged to cool the blood passing through it. He then says, "We observe, in the first place, that it is probable that the albuminous juice exuding from the impregnated uterus is stored with no small abundance of aerial substance, as may be observed from its white colour and frothy character [Needham's uterine milk]. And in further indication of this, the primogenial juices of the egg, which have a great resemblance to the seminal juice of the uterus, appear to abound in air particles. For if the white or the yolk of an egg be put into a glass from which the air is exhausted by the Boylian pump these liquids will immediately become very frothy and swell up into an almost infinite number of little bubbles and into a much greater bulk than before—a sufficiently clear proof that certain aerial particles are most intimately mixed with these liquids. To which I add that the humours of an egg when thrown into the fire, give out a succession of explosive cracks which seem to be caused by the air particles rarefying and violently bursting through the barriers which confined them. Hence it is that the fluids of the egg are possessed of so fermentative a nature. For it is indeed probable that the spermatic portions of the uterus and its carunculae are naturally adapted for separating aerial particles from arterial blood. These observations premised, we maintain that the blood of the embryo, conveyed by the umbilical arteries to the placenta or uterine carunculae, brings not only nutritious juice, but along with this a portion of nitro-aerial particles to the foetus for its support, so that it seems that the blood of the infant is impregnated with nitro-aerial particles by its circulation through the umbilical vessels quite in the same way as in the pulmonary vessels. And therefore I think that the placenta should no longer be called a uterine liver but rather a uterine lung". These splendid words, informed by so much insight and scientific acumen, show that, by the time of Mayow, chemical embryology had definitely come into being. He died at the early age of thirty-six, and we may well ponder how different the subsequent course of this kind of study would have been if he had lived a little longer.

The second part of Mayow's treatise is concerned with respiration in the hen's egg during its development, and it may be noted that his observations on the air contained in the liquids before development probably account for the facts which have been reported at one time and another concerning an alleged anaerobic life of embryos in early stages. Mayow is wrong in supposing that the gas which he pumped out from white and yolk was purely "nitro-aerial", but he shows the greatest good sense in his reminder that the amount of nitro-aerial particles required by embryos must be comparatively small owing to their small requirement for "muscular contraction and visceral concoction". His remarks on the effect of heat on the developing egg are not so clear as the remainder of the treatise, but he seems to mean that the heat will disengage the nitro-aerial particles from the liquids, and so aid in respiration, an idea which was later used by Mazin. His fundamental mistake here was that he failed to realise that the egg-shell was permeable to air; and this vitiates all his reasoning about the respiration of the egg. "It will not be irrelevant", he says, "to enquire here whether the air which is contained in the cavity in the blunter end of every egg contributes to the respiration of the chick." He first notes that the cavity in question lies between two membranes and not between the shell-membrane and the shell as Harvey himself had supposed; and then he goes on to say that he disagrees with the opinion of Fabricius, who had asserted that the air in the air-space serves for the respiration of the chick. His reasons are (1) that there would not be enough therein for the needs of the embryo which would use it, as it were, in one gulp, and (2) that the air in it cannot pass through the inner membrane, an error into which he was led by observing that, if an egg-shell with its contents removed and its air-space intact, was put into a vacuum, the air-space would swell up until it was as big as the egg itself. Mayow sees now what had escaped the attention of all previous observers, namely, that the egg-contents are not "rarefied or expanded, but are on the contrary condensed and forced into a narrower space than before". Such a condensation could, he thinks, take place in four ways, (*a*) by an increase in propinquity of discrete particles, (*b*) by a subsidence of motion on the part of a congregation of particles into rest, (*c*) by the extraction of some subtle spirit from amongst the particles, and, (*d*) by a decrease in elasticity on the part of some elastic substance previously present. We should at the present time choose the third alternative as being the truest, in view of the loss of water and carbon dioxide which the egg suffers as it develops, but Mayow chose the fourth, thinking it probable that the "air distributed among the juices of the egg loses its elastic force on account of the fermentation produced among these juices by incubation". Now since the egg-contents are compacted into smaller bulk by the process of incubation, a vacuum would be created somewhere if Nature had not, with her customary prudence, inserted a small amount of air into the air-space which might in due course expand and avoid this. His proof for this was an inaccurate observation; he thought he saw, in eggs at a late stage, when the contents were removed, the air-space collapse to the normal size which it occupies in unincubated eggs. He expressly says that his theory does not depend upon the conception of *horror vacui*, but that, by the compressive action of the imprisoned air, the fluids of the egg would be forced into the umbilical vessels, and the particles composing the embryonic body packed more tightly together. "The internal air appears to perform the same work as the steel plate bent round into numerous coils by which automata are set in motion."

With this ingenious but erroneous supposition Mayow concludes what is undoubtedly the first great contribution to physiological or biophysical embryology. His views on foetal respiration were soon generally accepted, as the writings of Zacchias, Viardel, Pechlin and John Ray show, but Sponius as late as 1684 was asserting that the lungs of the foetus were functional *in utero*, absorbing from the amniotic liquid the nitro-aerial particles which P. Stalpartius supposed the placenta to be secreting into it. It is interesting to note that by Mayow's own air-pump method Bohn found nitro-aerial particles in the uterine milk in 1686, and Lang found them in the amniotic liquid in 1704. The problem had by then arrived at a stage beyond which it could not progress in the absence of quantitative methods.

The year 1675 saw the publication of Nicholas Hoboken's useful treatise on the anatomy of the placenta, and of the English edition of P. Thibaut's *Art of Chymistry*. I mention the latter here, because of a reference to the special conditions of embryonic life which is found in it. As yet no real help was being given to embryology by contemporary chemistry.

The Magistery and Calx of Egg-shells.

Obs. 2. That you must use the eggshells of hens and not of ducks, geese, or turkeys because that hens eggshells easier calcin'd being thinner by reason that a hen is a more temperate animall; waterfowl are hotter and by reason of their heat do concoct and harden their eggshells more than other fowl; and from thence it comes that you must have a greater quantity of your Dissolvant, employ more heat, and spend more time to calcine the eggs of waterfowl than those of hens.

About this time also Francis Willoughby published his famous book on birds, an attempt to bring Aldrovandus up to date, in which a good picture is given of the embryological knowledge of the time, although no new observations or theories are given. Another contemporary review is that of Barbatus.

In 1677, spermatozoa were discovered, as announced by Hamm and Leeuwenhoek in the *Philosophical Transactions of the Royal Society*, though Hartsoeker afterwards claimed that he had seen them as early as 1674, but had not had sufficient confidence to publish his results. There is a reference to this in the letters of Sir Thomas Browne, who, writing to his son, Dr Edward Browne, on December 9, 1679, said, "I sawe the last transactions, or philosophicall collections, of the Royal Society. Here are some things remarkable, as Lewenhoecks finding such a vast number of little animals in the melt of a cod, or the liquor which runnes from it; as also in a pike; and computeth that they much exceed the number of men upon the whole earth at one time, though hee computes that there may bee thirteen thousand millions of men upon the whole earth, which is very many. It may bee worth your reading".

At the same time as these events were taking place, Robert Boyle, at Oxford and London, was engaged in carrying out those experiments in chemistry which led him before long to write his *Sceptical Chymist.* It is not generally known that in this work, which appeared in 1680, and which set the key for the whole spirit of subsequent physico-chemical research, Boyle has a reference to embryology, and, curiously enough, in connection with a point which, although it is easily seen to be of the highest importance, has been quite overlooked by the commentators upon him. One of the main things he was trying to urge was that, until some system could be proposed which would give a means of quantitative estimation of the constituents of a mixture, no further progress would be made. He was asking, in fact, that chemistry should become an exact science, and his demand is only veiled by the unfamiliarity of his language. His preference for the "mechanical or corpuscularian" philosophy was mainly due to his realisation that, unless chemistry was going to start measuring something, it might as well languish in the obscurity to which Harvey would have willingly relegated it. Thus he says, "But I should perchance forgive the Hypothesis I have been all this time examining (that of the alchemists), if, though it reaches but to a very little part of the world, it did at least give us a satisfactory account of those things which 'tis said to teach. But I find not that it gives us any other than a very imperfect information even about mixt bodies themselves; for how will the knowledge of the *Tria Prima* discover to us the reason why the Loadstone drawes a Needle, and disposes it to respect the Poles, and yet seldom *precisely* points at them? how will this hypothesis teach us how a Chick is formed in the Egge, or how the seminal principles of mint, pompions, and other vegetables, can fashion Water into various plants, each of them endow'd with its peculiar and determinate shape and with divers specifick and discriminating Qualities? How does this hypothesis shew us, *how much* Salt, *how much* Sulphur, *how much* Mercury must be taken to make a Chick or a Pompion? and if we know that, what principle is it, that manages these ingredients and contrives, for instance, such liquors as the White and Yolke of an Egge into such a variety of textures as is requisite to fashion the Bones, Arteries, Veines, Nerves, Tendons, Feathers, Blood and other parts of a Chick; and not only to fashion each Limbe, but to connect them altogether, after that manner which is most congruous to the perfection of the Animal which is to consist of them? For to say that some more fine and subtile part of either or all the Hypostatical Principles is the Director in all the business and the Architect of all this elaborate structure, is to give one occasion to demand again, *what proportion and way of mixture* of the *Tria Prima* afforded this Architectonick Spirit, and what Agent made so skilful and happy a mixture?" Boyle's instance of the magnetic needle pointing nearly, not exactly, at the north, and his use of the expressions "how much, how many, proportion, way of mixture", indicate that he was moving towards a quantitative chemistry, and by express implication a quantitative embryology. Elsewhere he says that he thinks the *Tria Prima* will hardly explain a tenth part of the phenomena which the "Leucippian" or atomistic hypothesis is competent to deal with. Thus, although Boyle made few experiments or observations on embryos, he occupies a very important position in the history of embryology.

During the last two decades of this century, the Oxford Philosophical Society were occupied on a good many occasions with problems relating to embryology. It is extremely interesting to note, in connection with what we have just seen in Boyle, that John Standard of Merton College reported on February 10, 1685, "the following obbs. concerning ye weight of ye severall parts of Henn's eggs; done with a pair of scales which turned with ½ a grain.

	ozs.	dr.	scr.	grns.
A henn's egg weighed	2	–	1	15
The skin weighed	–	–	–	16
The shell	–	2	2	4
The yolk	–	5	1	–
The white	1	1	–	6
Loss in weighing				9

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	ozs.	dr.	scr.	grns.
Another raw egg of the same sort	2	1	2	13
Another	2	1	1	19
The former egg boiled	2	1	1	18
Lost in boiling	–	–	–	15
The skin	–	–	–	13
The shell	–	1	2	19
The yolk	–	5	–	7
The white	1	2	–	13
Loss in weighing				5 ”

Another early quantitative observation was that of Claude Perrault who found about 1680 that developing ostrich eggs lost one-ninth of their weight in five weeks. The Oxford Philosophical Society, however, preferred as a rule to consider more unusual things, such as "the egges of a parrot hatched in a woeman's bosome, a hen egg figur'd like a bottle, a hen egg that at the big ende had a fleshie excrescence, another hen-eg, monstrous, a suppos'd cocks egg, and the eggs of a puffin, an elligug, and a razor-bill". Mention of these different kinds of eggs reminds us that the systematic collection and classification of eggs had been begun some years before by Sir Thomas Browne (as may be seen in John Evelyn) and by John Tradescant. About this time R. Waller made some noteworthy observations on the "spawn of frogs and the production of Todpoles therefrom", extending the work begun by Swammerdam not long before. Mauriceau now gave a description of the phenomenon of sterile foetal atrophy. The century fittingly closes with Michael Ettmüller's ponderous treatise, in which all the embryological work of the seventeenth century is summarised with considerable accuracy. He supported the moribund menstruation theory of embryogeny with the argument that animals do not menstruate because they are more prolific than men, and therefore all their blood is required for generation. Garmann's *Oologia curiosa*, which appeared in 1691, is worth mention also, as a review of the knowledge of the time. But that his work was what the booksellers' catalogues describe as "curious" is shown by the following chapter-headings: *De ovo mystico, mythico, magico, mechanico, medico, spagyrico, magyrico, pharmaceutico.*

3·9. The Theories of Foetal Nutrition

During the course of the seventeenth, and the first quarter of the eighteenth, century, many theories were propounded concerning foetal nutrition. It is convenient to classify them.

I. That the embryo was nourished by the menstrual blood.

- Beckher, 1633.
- Plempius, 1644. (He did not deny that the umbilical cord was functional, but insisted that the blood passing through it was menstrual.)

In 1651 Harvey's work was published.

- Sennertus, 1654.
- Seger, 1660.
- van Linde, 1672.
- F. Sylvius, 1680.
- Cyprianus, 1700.

II. That the embryo was nourished by its mouth.

(*a*) By the amniotic liquid.

(A) In addition to the umbilical blood.

- Harvey, 1651.
- W. Needham, 1667.
- de Graaf, 1677.
- C. Bartholinus, 1679.
- van Diemerbroeck, 1685.
- Ortlob, 1697.
- D. Tauvry, 1700.
- Linsing, 1701.
- Pauli, 1707.
- Barthold, 1717.
- S. Middlebeek, 1719.
- Teichmeyer, 1719.
- Gibson, 1726.

(B) Alone; the umbilical blood being regarded as unnecessary or of minor importance.

- Moellenbroeck, 1672.
- Cosmopolita, 1686.
- Everardus, 1686.
- P. Stalpartius, 1687.
- Bierling, 1690.
- Case, 1696. (Case thought the embryo arose entirely out of the amniotic liquid like a precipitate from a clear solution.)
- Berger, 1702.

These persons referred as their principal experimental basis to cases in which embryos had been born without umbilical cords, e.g. of those of:

- Rommelius, 1675 (in Velsch).
- Valentinius, 1711.

(*b*) By the uterine milk or *succum lacteo-chylosum*.

- Mercklin, 1679.
- Drelincurtius, 1685.
- Bohnius, 1686.
- Zacchias, 1688.
- Tauvry, 1694.
- Franc, 1722.
- Dionis, 1724.

III. That the embryo was nourished through the umbilical cord only.

(*a*) By foetal blood (the circulations distinct).

- Arantius, 1595.
- Harvey, 1651.
- W. Needham, 1667.
- F. Hoffmann, 1681. (He proved the point by injection long before Hunter, who is stated by Cole to have been the first to demonstrate this.)
- Ruysch, 1701.
- Snelle, 1705.
- Falconnet, 1711.

It is to be noted that Bierling, P. Stalpartius, Berger, Barthold, and Charleton, who supported the discontinuity theory of the circulations, were all upholders of the theory of foetal nourishment *per os*, so that their reasons for doing so were not those on account of which we agree with Hoffmann and Needham at the present time.

(*b*) By maternal blood (the circulations continuous).

- Laurentius, 1600.
- de Marchette, 1656.
- Rallius, 1669.
- Muraltus, 1672.
- Blasius, 1677.
- Veslingius, 1677.
- Hamel, 1700.
- de Craan, 1703.
- Lang, 1704.
- van Horne, 1707.
- Freind, 1711. (Freind's *Emmenologia* deserves a special mention. He proved by a calculation that the amount of blood passing through the umbilical cord would be sufficient for the needs of the embryo. This is a parallel to Harvey's famous calculation about the circulation of the blood. He also quotes some experiments of

Rayger and Gayant, who injected a blue dye into the foetal circulation and found it again in the maternal. Therefore he regards it as continuous.)

Mery, 1711. (Mery combated Falconnet's view of the separate circulations. He said that he had not himself tried Falconnet's experiment, but that some students had, and could not repeat it.)

Aubert, 1711. (Narrative of a case in which the umbilical cord had not been tied at the maternal end and the mother had nearly bled to death through it.)

Nenterus, 1714.

Wedel, 1717.

Bellinger, 1717. (Bellinger believed that the maternal blood was transformed by the embryonic thymus gland into proper nourishment for itself, after which it was secreted into the mouth by the salivary ducts and so went to form meconium without the necessity for deglutination. Heister's comments on this extraordinary theory are worth reading. Perhaps Bellinger was indebted to Tauvry for his idea of the importance of the thymus gland. Tauvry had drawn attention in 1700 to its diminution after birth.)

de Smidt, 1718.

Dionis, 1724.

(*c*) By menstrual blood.

Plempius, 1644.

(*d*) By uterine milk.

Ent, 1687.
Camerarius, 1714. (*Opinio conciliatrix!*)
F. Hoffmann, 1718.

(*e*) By the amniotic fluid.

Vicarius, 1700.
Goelicke, 1723.

IV. That the embryo was nourished by pores in its skin.

Deusingius, 1660.
Nitzsch, 1671.
Stockhamer, 1682.

This was suggested on the ground that in the earlier stages of development there is no umbilical cord. In 1684 St Romain argued against it on the ground that, if it were true, the embryo would dissolve in the amniotic liquid.

During this period also there were continued disputes about the origin of the amniotic liquid. van Diemerbroeck and Verheyen considered that it could not be the sweat of the embryo, for the embryo was always much too small to account for it, and, moreover, Tertre had described cases where the secundines had been formed with the membranes but in the absence of the embryo. Dionis affirmed that, whatever it was, it could not be urine, for urine will not keep good for nine days, *a fortiori* not for nine months. Drelincurtius put forward a theory that the embryo secreted it from its eyes and mouth by crying and salivating, while Bohn and Blancard derived it from the foetal breasts. Lang, Berger and Gofey criticised this notion without bringing forward anything constructive, and Gofey was in his turn annihilated by D. Hoffmann, who with Nenter and König supported the modern view, namely, that it was a transudation from the maternal blood-vessels in the decidua. The question was complicated further by the alleged discovery by Bidloo in 1685 of glands in the umbilical cord, and by Vieussens in 1705 of glands on the amniotic membrane. J. M. Hoffmann and Nicholas Hoboken supported the view that these were the important structures. There the problem was left during the eighteenth century, various writers supporting different opinions from time to time, and it is still under discussion (see Section 22).

Very early in the eighteenth century (1708) there appeared a work by G. E. Stahl, van Helmont's most famous follower, which struck the keynote of the whole century. Stahl's *Theoria Medica Vera*, divided as it was into Physiological and Pathological sections, belonged in essence to the *a priori* school of Descartes and Gassendi. It differed from them profoundly, of course, for, instead of trying to explain all biological phenomena, including embryonic development, from mechanical first principles, it started out from first principles of a vitalistic order, and, having combined all the archaei into one informing soul, it sought to show how the facts could be perfectly well explained on this basis. But the spiritual kinship of Stahl with Descartes and Gassendi is due to an atmosphere which can only be called doctrinaire, and which was common to them all. Like the methodist school of Hellenistic medicine, they subordinated the data to a preconceived theory, during which process any awkward facts were liable to be rather submerged than subordinated.

In 1722 Antoine Maître-Jan published his book on the embryology of the chick, the only one on this subject between Malpighi and Haller. It was an admirable treatise, illustrated with many drawings which, though not very beautiful, were as accurate as could be expected at the time. Perhaps its most remarkable characteristic is its almost complete freedom from all theory—Maître-Jan says hardly a word about generation in general, and is far from putting forward a "system" in the usual eighteenth-century manner. He contents himself with the recital of the known facts, including those added by his own observations. He gives no references, and writes in an extremely modern and unaffected style.

The only traces of theoretical presupposition which can be found in him are Cartesian, for he speaks of the activity of ferments in blood-formation. He is an epigenesist, and long before Brooks, he gives the right explanation of Malpighi's error, affirming that the hot Italian summer was responsible for some development in Malpighi's eggs before Malpighi examined them. Maître-Jan's book must have been accessible both to Buffon and Haller, so it is difficult to see why they should have perpetuated Malpighi's mistake till nearly the end of the century.

In technique, Maître-Jan was pre-eminent. He was the first embryologist to make practical use of Boyle's suggestion regarding "distilled spirits of vinegar" for hardening the embryo so that it could be better dissected. He also used "weak spirits of vitriol"; after treating blastoderms with it, he said, "I saw with pleasure an infinity of little capillary vessels which had not appeared to be there before". He made a few chemical experiments also, noting that vinegar would coagulate egg-white, and estimating quantitatively the difference in oil-content of different yolks—though for this he gives no figures.

His theory he relegated to an appendix entitled *Objections sur la génération des animaux par de petits vers.* There were sixteen of them, but the most cogent one was that, as little worms had been found under the microscope in pond-water, vinegar, and all kinds of liquids, there was no reason to suppose that those in the semen were in any essential way connected with generation. For his time, this argument was an excellent one, and was open to no demur save on the ground of filtration experiments which had not yet been made (see p. 215).

About this time there was some controversy over the circulation of blood, the *foramen ovale*, etc., in the embryo. From 1700 to 1710, Tauvry and Mery were engaged in a polemic on this subject, and the latter also corresponded with Duverney, Silvestre and Buissière in a controversy which recalls that of Laurentius and Petreus a hundred years before. Nicholls wrote later on the same subject. Daniel Tauvry was interesting, however, for other reasons. He was an epigenesist, and wrote vigorously against the view that the soul constructed during embryogeny a suitable home for itself.

Nine years later two books appeared, which form very definite landmarks in the history of embryology. One was Martin Schurig's *Embryologia*, and the other the *Elementa Chymiae* of Hermann Boerhaave.

The former, however, gave to the world no new experiments or observations; it was the first of what we should now call the typical "review" kind of publication. Schurig saw that he was living at the end of a great scientific movement following the Renaissance, and set himself accordingly for many years to compile large treatises on definite and restricted subjects, taking care to give all references with meticulous accuracy, and to omit no significant or insignificant work. His *Spermatologia* was the first to appear (in 1720), and it was followed in 1723 by *Sialologia* (on the saliva), *Chylologia* (1725), *Muliebria* (1729), *Parthenologia* (1729), *Gynaecologia* (1731) and *Haematologia* (1744). His *Embryologia* was the last but one of the series. In it he treated compendiously of all the theories which had been advanced about embryology during the immediately preceding two centuries, and his chapters on foetal nutrition and foetal respiration throw a flood of light on to the "intellectual climate" in which Harvey and Mayow worked, providing, as it were, the perishable background of their immortal thoughts. Schurig's bibliography is a very striking part of his book, extending to sixteen pages, and including five hundred and sixty references; it was the first attempt of its kind.

3·10. Boerhaave, Hamberger, Mazin

Hermann Boerhaave was a more prominent figure, a Professor at Leyden for many years, and renowned for his encyclopaedic learning on all subjects remotely connected with medicine. His *Elementa Chymiae*, which became the standard chemical book of the whole period, demonstrates throughout the exceedingly wide outlook of its author, and contains in the second volume what must be regarded as the first detailed account of chemical embryology. I reproduce here the relevant passages in full because of their great interest. It will be noted that they are cast in the form of lecture addresses, as if they had been taken down direct from the lectures of the Professor, a fact which gives them a peculiar charm when it is remembered how many great men must have listened to them, among them Albrecht von Haller and Julien de la Mettrie. In considering what follows, it should be noted that Boerhaave's interest is biological all the time, and that he does not treat the liquids of the egg, as nearly all the chemists before him had done, as substances of curious properties indeed, but quite remote from any question relating to the development of the embryo. Another interesting point is that he deals only with the white, and hardly mentions the yolk; this is perhaps to be explained by the Aristotelian theory that the embryo was formed out of the white, and only nourished by the yolk (*ex albo fieri, ex luteo nutriri*), a theory which was still alive, in spite of Harvey, in the first half of the eighteenth century. If this was what was at the bottom of Boerhaave's mind, then it is obvious that the egg-white would be to him the liquid inhabited more particularly by the plastic force. This, then, is what he has to say about the biochemistry of the egg.

Op. Chem. in Animalia. [Processus 109.] *The albumen of a fresh egg is not acid, nor alkaline, nor does it contain a fermented spirit.* The white of a fresh egg, separated from the shell, the membranes, and the yolk, I enclose in clean glass vessels, and into each of these I pour different acids, and shake them up, mixing them, and no sign of ebullition appears however I treat them. Therefore I lay these vessels aside. Now in these other two vessels I have two fresh portions of albumen, and I mix with them in one case alkaline salt and in the other volatile alkali. You see they are quiet without any sign of effervescence. Now behold a remarkable thing, in this tall cylindrical vessel is half an ounce of the albumen of an egg and two drams of spirits of nitre, in this other vessel is half an ounce of egg-white, together with four and a half ounces of oil of tartar *per deliquium* both heated up to 92 degrees. Pray observe and behold, with one movement I pour the alkaline albumen into the acid albumen, with what fury they boil up, into what space they rarefy the mass, so that they stream out of the vessel although it is ten pints in size [*decupli capace*]. They have scarcely changed their colour. But when the effervescence has abated how suddenly they return to the limits of space occupied before. But now if more egg-white is heated to 100 degrees in a retort [*cucurbita*] an insipid water containing no spirit is given off. If egg-white is applied to the naked eye or naked nerve it does not give the smallest sense of pain, and scarcely affects the smell; nothing more inert and more insipid can be put on the tongue. It appears mucous and viscid to the touch, not at all penetrable. Hence in the fresh white of an egg there is no alkali or acid, or both together. It is indeed a thick, sticky, inert, and insipid liquor, yet from this truly vital liquid at a heat of 93 degrees within the space of 21 days the chick grows in the incubated egg from a tiny mass hardly weighing a hundredth of a grain into the perfect body of an animal, weighing an ounce or more. We have learnt therefore of a liquid distinct from all others, from which by inscrutable causes fibres, membranes, vessels, entrails, muscles, bones, cartilages, and all the other parts, tendons, ligaments, the beak, the claws, the feathers, and all the humours can be produced—and yet in this liquid we find softness, inertia, absence of acid, alkali, and spirit, and no tendency to effervesce. Indeed, if there were the slightest effervescence in it, it would certainly break the eggshell, therefore we see from how slow and inactive a mass all the solid and fluid parts of the chick are constructed. And yet this itself is rendered absolutely useless for forming the chick by greater heat. It scarcely bears 100 degrees with good effect but at a less temperature never brings forth a chick, for under 80 degrees will not suffice. But by a heat kept between these limits, there is brought about so marvellous an attenuation of the mucous inactivity that it can exhale a great part through the shell of the egg and the two membranes, the yolk and chalazae alone remaining along with the amniotic sac. For the yolk, the uterine placenta of the chick, takes little part in the nourishment. Meanwhile Malpighius has shown that this albumen is not a liquid of a homogeneous kind, as the blood-serum flowing through the vital vessels is, but that it is a structure composed of numerous membrane-like and distinct small saccules, filled with a liquid of their own, in the same way as in the vitreous humour of the eye.

[Processus 111.] *Exploration of the egg-white with alcohol.* In this transparent vessel is the albumen of an egg, and into it, as you perceive, I gently pour the purest alcohol, so that it descends down the sides of the vessel and reaches the albumen. I do this deliberately and with such solicitude that you may see the surface of the albumen which, touching the alcohol, holds it up, being immediately coagulated, while the lower part remains liquid and transparent. As I now gently shake them together, it appears evident that wherever the alcohol touches the albumen a concretion is formed. Behold now, while I shake them up thoroughly together, all the egg-white is coagulated. If alcohol previously warmed is employed in this experiment, the same result is brought about but more rapidly. It appears therefore that the purest vegetable spirits immediately coagulate the plastic and nutrient material.

[Processus 112.] *The fresh albumen of an egg is broken up by distillation.* These fresh eggs have been cooked in pure water till they became hard. I now take the shining white, separating off all the other things, and break it up into small pieces. I put these, as you see, into a clean glass retort [*cucurbita*] and I duly cover it by fitting on an alembic and add a receiver. By the rules of the (chemical) art I place the whole retort in a bath of water and I apply to it successive degrees of fire until the whole bath is boiling. No vaporous streaks [*stria*] of spirits are given off but simple water in dewy drops and this in incredible quantity, more than nine-tenths. I continue so with patience until by the heat of boiling water no more drops of this humour are given off. Then this water shows no trace of oil, salt, or spirit; it is perfectly transparent and tasteless, except that it eventually grows rather sour. It is odourless, save that towards the end it gives off a slight smell of burning. It shows absolutely no sign of the presence of any alkali, when I test it in every way, as you can see for yourselves; nor does it reveal any trace of acid, when tried how you will. Here you see pounds of this water, but in the bottom of the now open retort see, I beg of you, how little substance remains. Behold, there are fragments contracted into a very small space in comparison with the former quantity. They are endowed with a golden yellow colour, especially where they have touched the glass, but yet they are transparent after the manner of coloured glass. When I take them out I find them very light, very hard, quite fragile, and breaking apart with a crack, smelling slightly of empyreuma, with a taste rather bitter from the fire, and without any flavour of alkali or acid. This is the first part of the analysis. Now I take these remaining fragments in a glass retort [*retortam*] in such a way that two-thirds remain over. I put the retort into a stove of sand, first arranging a large receiver. Then thoroughly luting all the joints I distil by successive grades of fire and finally by the highest which I call *suppressionis*. There ascends a spirit, running in streaks [*striatim*] fat and oily, and at the same time, volatile salts of solid form everywhere on the walls of the vessel, rather plentiful in proportion to the dried fragments but small in proportion to the whole albumen before the water had been removed from it. Finally an oil appears besides the light golden material mixed with the first, black, thick, and pitchy. When by the extreme force of the fire this oil is finally driven forth, then the earth in the bottom, closely united with its most tenacious oil, swells up and is rarefied and rises right up to the neck of the retort so that had the retort been overfull it would have entered into the neck and clogged it up, even causing it to burst, with danger to the bystanders. The operation is to be continued till no more comes out. That first spirit, oily and fatty, is clearly alkaline by every test, as you may tell from the way it effervesces when acid is poured on it. If we rectify it we resolve it into an alkaline volatile salt, an oil, and inert foetid water. The salt fixed to the walls is completely alkaline, sharp, fiery, oily, and volatile; and the final oil is specially sharp, caustic, and foetid. The black earth which remains in the retort is shiny, light, thin, and fragile, foetid from the final empyreumatic oil, and soft because of it. If then it is burnt on an open fire, it leaves a little fixed earth which is white, insipid, tasteless, and odourless, from which scarcely any salt can be extracted, but only a very heavy dusty powder [*pollinem*].

Cf. the dry distillation of egg-white by Pictet & Cramer in 1919.

[Processus 113.] *The fresh albumen of an egg will putrefy.* Sound eggs kept at 70° for some days will become foetid and stink....We have learnt then that this is the nature of the material which will shortly be changed into the structure, form, and all the parts of the animal body. Repose and a certain degree of heat produce that effect in that material. We observe therefore the spontaneous corruption and change of the material, and what is extremely remarkable, if an impregnated egg is warmed in an oven [*in hypocaustis*] to a heat of 92 degrees it employs these attenuated parts changed by such a heat to nourish, increase, and complete the chick for 21 days. But in this chick nothing alkaline, foetid, or putrid is found, hence observe, O doctors [*medici*], the remarkable manifestations of nature—by repose and a certain degree of heat a thick substance becomes thin, a viscous substance becomes liquid, an odourless substance becomes foetid, an insipid substance becomes sour and extremely acrid and bitter to the taste, a soothing substance becomes caustic, a non-alkali becomes alkaline, a latent oil becomes sweet and putrid. Let these results be compared with the observations of Marcellus Malpighius on the incubated egg, and we shall observe things which shall surprise us. I took care to investigate only the albumen of the egg first of all, separating the other parts off where possible, for the albumen alone forms the whole of the material which proceeds to feed [*in pabulum*] the embryo. The other constituents of the egg only assist in changing the albumen, so that when it is changed, it may be applied to forming the structure of the chick.

Boerhaave's treatment of these subjects has only to be compared with that of Joachim Beccher, who wrote in 1703, to show how thoroughly modern in outlook it is. Beccher's *Physica Subterranea* contains a whole section devoted to the growth of the embryo, but it is extremely confused and very alchemical in its details. The advance made in the thirty years between Beccher and Boerhaave was immense, but, if the biochemistry of development advanced so fast, its biophysics was not far behind, as is shown by the work of G. E. Hamberger and J. B. Mazin.

Hamberger's most important contributions, contained in his *Physiologia medica* of 1751, were his quantitative observations on the water-content of the embryo and its growth-rate, in which he had no forerunners. Hamberger showed "that there are much less solid parts in the foetus than in the adult. The cortical substance of the brain of an embryo loses 8694 parts in 10,000 on drying but in the adult it only loses 8096 and that of the cerebellum from 81 parts is reduced to 12. The maxillary glands of the embryo lose out of 10,000 parts 8469, the liver 8047, the pancreas 7863, the arteries 8278 and even the cartilages lose four-fifths of their weight, decreasing from 10,000 to 8149½". The corresponding figures for the adult were: liver 7192, and heart 7836. These figures do not widely differ from those obtained in recent times.

J. B. Mazin published his *Conjecturae physico-medico-hydrostaticae de respiratione foetus* in 1737 and his *Tractatus medico-mechanica* in 1742. In the first of these works Mazin supports what is essentially Mayow's theory of embryonic respiration, without, however, mentioning Mayow more than once. It had not been popular since 1700, though Pitcairn had defended it. Mazin put the liquids of eggs under an air-pump, and observing that air could be extracted from them affirmed that the air was hidden in them and that the embryo could therefore respire. He spoke of "aerial particles" in the amniotic liquid, and discussed the respiration of fishes in connection with this. The specific gravity of the embryo also interested him, and he did a great deal of calculation and experiment on it. His most interesting passage, perhaps, is that in which he mentions the "Eolipile" of the Alexandrians, the primitive form of the steam-engine, and says that just as the heat of the fire makes the water boil, so the heat of the viscera makes the amniotic liquid boil, giving off respirable vapours. The time-relations of this analogy are interesting, for in 1705 Thomas Newcomen had succeeded in making a steam-engine which worked with considerable precision, and the question of steam-power was widely discussed. Possibly Mazin was acquainted with the Marquis of Worcester's *Century of the Names and Scantlings of Inventions*, which had been published in 1663, and which had contained an aeolipile or "water-commanding machine". England was the centre of this movement and other countries employed Englishmen as engineers; Humphrey Potter, for instance, erected a steam-engine for pumping at a Hungarian mine in 1720.

As for the discovery of oxygen, it was near at hand, and Scheele in 1773 and Priestley in 1774 were soon to supply the knowledge without which Mazin could not proceed further.

In his second book, Mazin reported many quantitative observations on the specific gravity of the embryo. He found that it diminished as development proceeded, being to the amniotic liquid as 282 to 274 in the fourth month and as 504 to 494 in the fifth month.

Another instance of the way in which experimental physical questions now began to come in is afforded by the work of Joseph Onymos, whose *De Natura Foetu* of 1745 spoke of the specific gravity of the embryo at different stages of development.

These writers, together with Haller himself, and J. C. Hefter who handled problems of embryonic rate of growth contribute to one of the best, because most quantitative, aspects of eighteenth-century embryology.

3·11. Albrecht von Haller and his Contemporaries

Boerhaave's greatest pupil was Albrecht von Haller. Like O. W. Holmes, at Harvard, Haller occupied a "settee" rather than a "chair", at Göttingen, and taught not only physiology but also medicine and surgery, botany, anatomy and pharmacology. Nor did he merely deal with so many subjects superficially; in each case he published what amounted to the best and most complete text-book up to then written. Haller was made Professor in 1736, and for many years worked at Göttingen, devoting much of his time to embryological researches, which, with those of his opponent Wolff, stand out as the greatest between Malpighi and von Baer. In 1750 he published a series of dissertations and short papers on all kinds of physiological subjects, which would have been the direct ancestors of the modern compilations of groups of experts, had they been more systematically arranged. The volume on generation repays some study. The contributions relevant to the present discussion had been written at various times during the previous seventy years, and may be summarised as follows:

IV. Christopher Sturmius, *De plantarum animaliumque generatione.* (First published 1687.) In this paper Sturmius argues on behalf of the preformation theory "which in our times does not lack supporters", quoting Perrault, Harvey and Descartes. He contents himself with countering arguments which had been urged against it, as, (*a*) spontaneous generation, (*b*) annual recurrence of plants, (*c*) insect metamorphosis, (*d*) generation without copulation.

V. Rudolf Jacob Camerarius, *Specimen experimentorum physiologico-therapeuticorum circa generationem hominis et animalium.* The most interesting thing about this is that Camerarius mentions the observations of D. Seiller, a sculptor, who had ascertained that the body is five times the size of the head in the embryo but seven and a half times the size of it in the adult. This is in the direct line between Leonardo and Scammon.

XV. Philip Gravel, *De Superfoetatione.* (First published 1738.)

XVIII. Adam Brendel, *De embryone in ovulo ante conceptum praeexistante.* (First published 1703.) Brendel "stands for the Graafian hypothesis". Unfortunately, he was also a preformationist and believed that every limb, organ, and function existed not potentially but actually in the unfertilised egg before its passage down the Fallopian tube.

XXII. Camillus Falconnet, *Non est fetui sanguis maternus alimento.* (First published 1711.) This is the first of the French contributions to the book; they are all very markedly shorter than the German ones and much less heavily ornamented with irrelevant quotations. Falconnet is concerned to prove that the maternal and foetal circulations are separate, and he describes in an admirably concise manner an experiment in which he bled a female dog to death, after which, opening the uterus, he discovered that the embryonic blood-vessels were full of blood although those of the mother had none in at all. Arantius was therefore justified. Falconnet was soon confirmed by Nunn.

XXIII. Jean de Diest's *Sui Sanguinis solus opifex fetus est* (first published 1735) was written to prove a similar point. He refers to the experiment of Falconnet and the injections of F. Hoffmann, and criticises Cowper's experiment in which mercury had been injected into the umbilical vessels and found in the maternal circulation, on the grounds that mercury is so "tenuous and voluble" that it might pass where blood could not pass normally. He also objects to the view that the foetus is nourished by the amniotic liquid.

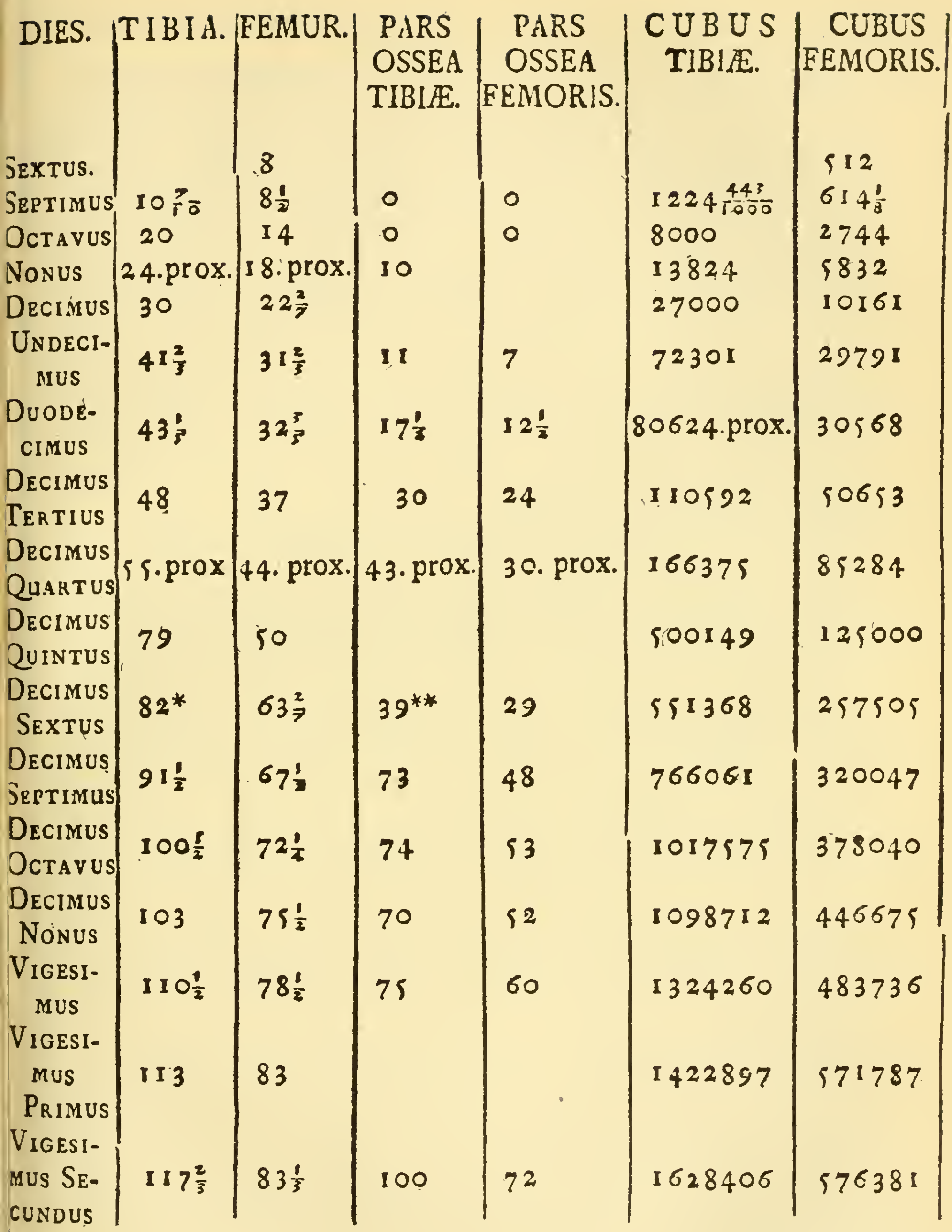
XXIV. Francis David Herissant, *Secundinae fetui pulmonis praestant officia, et sanguine materno fetum non alitur.* (First published in 1741.) An excellent paper, in which the respiratory function of the placenta is proved by the observation that the foetal blood-vessel leading to the placenta is always full of dark venous blood, while that leading away from the placenta is light and arterial [*floridiori coccineoque colore, ut ipsemet observavi*]. Herissant adduces also the cases of acephalic monsters, such as that of Brady, which could not possibly have drunk up any amniotic fluid, and yet were fully formed in all other respects. He concludes that the umbilical cord serves for respiration and nutrition.

XXV. After these three French workers, there is a great drop to Johannes Zeller, whose *Infanticidas non absolvit nec a tortura liberat pulmonum infantis in aqua subsidentia* (first published 1691) is a long-winded discussion of the floating lung test in forensic medicine. His memory deserves a word of obloquy for his vigorous insistence upon torture and death for infanticide even during puerperal insanity. Perhaps it was Zeller who called forth the noble answer of de la Mettrie to this inhumanity in his *Man a Machine*.

XXVI. Zeller's *De Vita Humana ex fune pendente* (first published 1692) is no better, though at the time, perhaps because of its striking title, it was famous. It deals with the ligation of the umbilical cord at birth.

This completes the list of the papers published by Haller in his 1750 collection. He retired from the Göttingen chair three years later, and in 1757 the first volume of his *Elementa Physiologiae* was published, probably the greatest text-book of physiology ever written. It appeared only by slow degrees, so that it was not until 1766 that the embryological section was available. This volume contains a discussion of a mass of literature, most of which had arisen during the preceding twenty-five years, for, although many of the names mentioned by Haller occur also in Schurig, yet many are quite new.

Haller himself published in 1767 a volume of his collected papers on embryology, most of which were concerned with the developing heart of the chick, which he worked out very thoroughly, in collaboration with Kuhlemann. (Kuhlemann had already done for the sheep what Harvey had done for the doe.) He made a beginning with the quantitative description of embryogeny, and one of his tables showing the changing lengths of the bones is reproduced herewith (Fig. 10). He was a convinced preformationist, a fact which was largely due to his researches on the hen's egg, where he observed that the yolk had a much more intimate connection with the embryo than had previously been supposed. Since the whole yolk was part of the embryo, as it were, the preformation theory seemed to him to fit the facts better than epigenesis.



DIES.	TIBIA.	FEMUR.	PARS OSSEA TIBIÆ.	PARS OSSEA FEMORIS.	CUBUS TIBIÆ.	CUBUS FEMORIS.
Sextus.		8				512
Septimus	$10\frac{7}{10}$	$8\frac{1}{2}$	0	0	$1224\frac{443}{1000}$	$614\frac{1}{8}$
Octavus	20	14	0	0	8000	2744
Nonus	24.prox.	18.prox.	10		13824	5832
Decimus	30	$22\frac{2}{7}$			27000	10161
Undecimus	$41\frac{2}{3}$	$31\frac{2}{3}$	11	7	72301	29791
Duodecimus	$43\frac{1}{5}$	$32\frac{3}{5}$	$17\frac{1}{2}$	$12\frac{1}{2}$	80624.prox.	30568
Decimus Tertius	48	37	30	24	110592	50653
Decimus Quartus	55.prox	44. prox.	43. prox.	30. prox.	166375	85284
Decimus Quintus	79	50			500149	125000
Decimus Sextus	82*	$63\frac{2}{7}$	39**	29	551368	257505
Decimus Septimus	$91\frac{1}{2}$	$67\frac{1}{3}$	73	48	766061	320047
Decimus Octavus	$100\frac{1}{2}$	$72\frac{1}{4}$	74	53	1017575	378040
Decimus Nonus	103	$75\frac{1}{2}$	70	52	1098712	446675
Vigesimus	$110\frac{1}{2}$	$78\frac{1}{2}$	75	60	1324260	483736
Vigesimus Primus	113	83			1422897	571787
Vigesimus Secundus	$117\frac{2}{3}$	$83\frac{1}{3}$	100	72	1628406	576381

Fig. 10. Facsimile of a table in A. von Haller's *Elementa Physiologiae* of 1766, containing some of his observations on the growth in length and weight of embryonic bones in the chick.

Haller went further than Schurig, in that he usually gave an opinion of his own after summarising those of other people, but his views were by no means always enlightened, and the atmosphere of Buffon is, on the whole, more congenial to us than that of Haller. Haller, for example, believed that the amniotic liquid had nutritious properties, and that the nutrition of the embryo in mammalia was accomplished first of all *per os* and afterwards *per umbilicum*. He denied that the placenta had any respiratory function, and, indeed, his whole teaching on respiration was retrograde. He mentions, however, an experiment of Nicolas Lemery's, in which it had been found that indigo would penetrate the shell of a developing hen's egg from the outside. Consequently, air might do so too, and Vallisneri had shown that, if an egg was placed in boiled water under an air-pump, the air inside would rush out through the shell and appear in the form of bubbles.

Haller was much more progressive in holding the origin of the amniotic liquid (according to him a subject of extraordinary difficulty—"*solutionem non promittam*") to be a transudation from the maternal blood-vessels. He followed Noortwyck in asserting the separateness of the maternal and foetal circulations in mammalia. He opposed the existence of eggs in vivipara—"We may conclude from all this", he said, "that the ovarian vesicles are not eggs and that they do not contain the rudiments of the new animal". But he accepted it in the restricted sense that the embryonic membranes resembled an egg, thus: "If we call an egg a hollow membranous pocket full of a humour in which the embryo swims, we may admit the opinion of the older authors who derive all animals from eggs with the exception of the tiny simple animals of which we have already spoken. It was in this sense that Aristotle and Empedocles before him, said that even trees were oviparous. This has also been confirmed by the experiments of Harvey on insects, fishes, birds, and quadrupeds".

Haller's most original work was in connection with the growth-rate of the embryo; here he struck out, for once, into entirely new country. "The growth of the embryo in the uterus of the mother is almost unbelieveably rapid. We do not know what its size is at the moment of its formation, but it is certainly so small that it cannot be seen even with the aid of the best microscopes, and it reaches in nine months the weight of ten or twelve pounds. In order to clear up this speculation, let us examine the growth of the chick in the egg. We cannot in this case either measure its size at the moment when the egg is put to incubate but it cannot be more than $\frac{4}{100}$ in. long, for if it were, it would be visible, and yet 25 days later it is 4 ins. long. Its relation is therefore as 64 to 64 millions or 1 to 1 million. This growth takes place in a singular manner, it is very rapid in the beginning and continually diminishes in speed. The growth on the first day is from 1 to 91⅛ and what Swammerdam calls a worm grows in one day from one-twentieth or one-thirtieth of a grain to seven grains, i.e. it increases its weight by 140 or 240 times. On the second day the growth of the chick is from 1 to 5, on the third day, from 1 to not quite 4, on the fifth day from 1 to something less than 3. Then from the sixth to the twelfth day, the growth each day is hardly from 4 to 5, and on the twenty-first day it is about from 5 to 6. After the chick has hatched, it grows each day for the first 40 days at an approximately constant rate, from 20 to 21 on each day. The increase of the first twenty-four hours is therefore in relation to that of the last twenty-four hours as 546¾ to 5 or 145 to 1. Now as the total increase in weight in the egg is to that of the whole growth period (up to the adult) as 2 to 24 ozs., all the post-embryonic growth is as 1 to 12, i.e. it is to the growth of one day alone early in incubation as 1 to 7½....The growth of man, like that of the chick, decreases in rapidity as it advances. Let us suppose that a man, at the instant of conception, weighs a hundred-thousandth of a grain and that a one-month old embryo weighs 30 grains; then the man will have acquired in that time more than 300,000 times the weight that he had to begin with. But if a foetus of the second month weighs 3 ozs. as it approximately does, he will only now have acquired 48 times the weight he had at the beginning of the period. This is a prodigious decrease in speed, and at the end of the ninth month he will not weigh more than about 105 ozs., which is not more than an average increase of 15 per month. A child three years old is about half the size of an adult. If then the adult weighs 2250 ozs. the three-year old child only weighs 281 ozs., which is an eighth of the adult weight. Now from birth to 3 years he will grow from 105 to 281 or as 5 to 14, but in the following 22 years he will only accumulate 2250 ozs. or eight times what he had at 3 years. The growth of a man will therefore be in the first month of intra-uterine life as 1 to 300,000, in the second as 1 to 48, in each of the others as 1 to 15. In the first 3 years of extra-uterine life his growth will be from 164 to 281 and in the succeeding 22 years from 281 to 384, and the growth of the first month to the last will be as 300,000 to $\frac{28}{456}$ or 136,800,000 to 28, or 4,885,717 to 1. The whole growth of man will consequently be as 108,000,000 to 1."

In spite of the rather unfamiliar language in which these facts are described, and the theory of the growth of the heart which Haller subsequently put forth to explain them, they remain fundamental to embryology. Their quantitative tone is indeed remarkably modern. In my opinion, when all the voluminous writings of Haller are carefully searched through, nothing more progressive and valuable than these figures can be found. Haller and Hamberger stand thus between Leonardo on the one hand and Minot and Brody on the other. That they stood so much alone is only another indication of the extraordinary reluctance with which the men of past generations assented to the truth contained in Robert Mayer's immortal words, "Eine einzige Zahl hat mehr wahren und bleibenden Wert als eine kostbare Bibliothek von Hypothesen".

Of development as a whole, Haller spoke thus, "In the body of the animal therefore, no part is made before any other part, but all are formed at the same time. If certain authors have said that the animal begins to be formed by the backbone, by the brain, or by the heart, if Galen taught that it was the liver which was first formed, if others have said that it was the belly and the head, or the spinal marrow with the brain, adding that these parts make others in turn, I think that all these authors only meant that the heart and the brain or whatever organ it was, were visible when none of the other parts yet were, and that certain parts of the embryonic body are well enough developed in the first few days to be seen while others are not so until the latter part of development; and others again not till after birth, such as the beard in man, the antlers in the stag, the breasts and the second set of teeth. If Harvey thought he descried an epigenetic development, it was because he saw first a little cloud, then the rudiments of the head, with the eyes bigger than the whole body, and little by little the viscera being formed. If one compares his description with mine, one will see that his description of the development of the deer corresponds exactly with mine of the development of the chick. If, more than twenty years ago, before I had made many observations upon eggs and the females of quadrupeds I employed this reasoning to prove that there is a great difference between the foetus and the perfect animal, and if I said that in the animal at the moment of conception one does not find the same parts as in the perfect animal, I have realised abundantly since then that all I said against preformation really went to support it". The reasons for this change of opinion become no clearer as Haller's writings are more assiduously read, and, as Dareste says, why he should have made it, will always remain a mystery.

The *emboîtement* aspect of preformation presented no difficulties to Haller. "It follows", he said, speaking of the generation of *Volvox*, "that the ovary of an ancestress will contain not only her daughter, but also her granddaughter, her great-granddaughter and her great-great-granddaughter, and if it is once proved that an ovary can contain many generations, there is no absurdity in saying that it contains them all."

The following passage is interesting. "We must proceed to say what is the efficient cause of the beautiful machine which we call an animal. First of all let us not attribute it to chance, as Ofrai [is this Julien Offray de la Mettrie? Haller had a habit of using Christian names, e.g. Turberville for J. T. Needham] would have us do, for although he pretends that all animals come from earth, he is not attached to the ancient opinion, and nobody now believes what Aelian says, namely that frogs are born from mud.... Vallisneri has found the fathers and mothers of the little worms in galls, a quest of which Redi despaired, and Redi in his turn has made with exactitude and precision those experiments which Bonannus, Triumphet, and Honoratus Faber had only sketched out imperfectly. Moreover, no seed, no clover.... This was the received opinion but in our century a proscribed notion has been revivified and some great men have pretended that there are little animals which are engendered by an equivocal generation, without father and mother, and that all the viscera and all the parts of these animals do not exist together, but that the nobler parts are formed first by epigenesis and that then the others are formed little by little afterwards." This is an admirable illustration of how spontaneous generation and epigenesis were bound up together. "M. Needham", Haller goes on to say, "does not admit an equivocal generation but he does admit epigenesis, and a corporeal non-intelligent force, which constructs a body from a tiny little germ furnishing the necessary matter for it. He says that there are only the primitive germs which were made at the original creation and that germs organised like animals do by no means pre-exist, for if they did, *molae uterinae*, encysted tumours, and the like, could not come into being." Haller then goes on to describe Needham's experiments with meat broths, etc., and objects to his "system", largely on the ground that "blind forces without any intelligence could hardly be able to form animals for ends foreseen and ready to take their places in the scheme of beings". He considers that Needham's theories are completely disproved by experiments such as those of Spallanzani, though, curiously enough, he does not quote the latter author in this connection. I shall return to this later.

"Nobody", he goes on to say, "has upheld epigenesis more than M. Wolff, who has undertaken an examination to demonstrate that plants and animals are formed without a mould out of matter by a certain constant force which he calls 'essential' [in his *Theoria Generationis*]....I have indeed seen many of the phenomena which he describes, and it is certain that the heart seems to be formed out of a congealed humour and that the whole animal appears to have the same consistency. But it does not follow that because this primitive glue which is to take on the shape of the animal does not appear to possess its structure and all its parts, it has not effectively got them. I have often given greater solidity to this jelly by the use merely of spirits of wine and by this means I saw that what had appeared to me to be a homogeneous jelly was composed of fibres, vessels, and viscera. Now surely nobody will say that the *vis essentialis* of the spirit of wine gave an organic structure to an unformed matter, on the contrary it is rather in the removal of transparency and the accession of greater firmness to the extremities, as well as the making of a more obvious boundary to the contour of a viscus that one could see the structure of a cellular tissue, which was ready to be formed but which the transparency had previously hidden and the wetness not allowed to be circumscribed by lines.... Finally, to cut a long story short, why does this *vis essentialis*, which is one only, form always and in the same places the parts of an animal which are so different, and always upon the same model, if inorganic matter is susceptible of changes and is capable of taking all sorts of forms? Why should the material coming from a hen always give rise to a chicken, and that from a peacock give rise to a peacock? To these questions no answer is given." This was the case because Wolff was not a theorist, but rather an experimentalist; his writings are marked by their abstention from the discussion of speculative points. The above passage is very interesting. It reminds us of the great difficulties with which the embryologists of this epoch had to contend. Serial section cutting was unknown, the staining of thin layers and reconstruction were unheard of; even the hardening of the soft embryonic tissues was only just discovered, as is indicated by Haller above. Hertwig has excellently discussed the advances in embryological technique which took place during this and the following century. It is true that dyes were beginning to be used, as some instances already given demonstrate, and as is seen from the use of madder in the staining of bones, which began about this time, and was later much used by the Hunters. Heertodt's *Crocologia* is important in this connection. Heertodt, by injecting saffron into the maternal circulation, found it afterwards in the amniotic fluid, and his experiment was cited by Haller in support of that theory of the origin of the liquid. But the most important advance in technique was the progress in artificial incubation. The art, though lost throughout the Middle Ages and the seventeenth century, was now to be revived.

During this period much work was done on it. As far back as 1600, de Serres had mentioned some experiments of this nature, but they were not successful. "The chicks", he said, "were usually born deformed, defective or having too many legs, wings, or heads, nature being inimitable by art." Birch, in his *History of the Royal Society*, also refers to it. "Sir Christopher Heydon [a relative of Digby's Sir John?] together with Drebell, long since in the Minories hatched several hundred eggs but it had this effect, that most of the chickens produced that way were lame and defective in some part or other." Antonelli states that similar trials were made at the court of the Grand-duke Ferdinand II at Florence about 1644, Thomas Bartholinus gives a like account with reference to the contemporary court of King Christian IV of Denmark, and Poggendorff and Antinori relate that the Accademia d. Cimento, inspired by Paolo del Buono, made trial of artificial incubation between 1651 and 1667.

But the most famous of all the attempts to make artificial as successful as natural incubation, were those of Réaumur, whose book *De l'art de faire éclore les poulets* of 1749 achieved a wide renown. He devotes many chapters to a detailed description of incubators of very various kinds: but he nowhere gives any indication of his percentage hatch. It was probably low. He speaks also of the "*funestes effets*" of the vapours of the dung on the developing embryos, without, however, furnishing any foundation for an exact teratology. In the second volume he describes those experiments on the preservation of eggs by varnishing them, which caught the imagination of Maupertuis and were held up to an immortal but by no means deserved ridicule by Voltaire in his *Akakia*. For the details of this amusing but irrelevant issue see Miall and Lytton Strachey.

After Réaumur, there were numerous continuations of the kind of work which he had done, in particular by Thevenot, La Boulaye, Nelli, Porta and Cedernhielm. Much the most interesting of these was the work of Beguelin, who attempted to incubate eggs with part of the shell removed so as to form a round window. He was not, however, successful in the carrying out of this very modern idea. Probably the most peculiar investigation made in this field at this time was that of Achard, who is mentioned in a passage of Bonnet's. "Réaumur did not suspect in 1749", says Bonnet, "that someday one would try to substitute the action of the electric fluid for his borrowed heat. This beautiful invention was reserved for M. Achard of the Prussian Academy who excels as an experimentalist. He has not so far succeeded in actually hatching a chick by means of so new a process, but he has had one develop up to the eighth day, when an unfortunate accident deranged his electrical apparatus." Bonnet goes on to say that this substitution of electricity for heat gives him hope that by electrical means an artificial fertilisation will one day become possible.

The references to these experiments and to those of many minor investigators will be found in Haller. By the beginning of the nineteenth century a great mass of literature had developed on the subject, and it had become possible to hatch out eggs more or less successfully from furnaces, though the losses were still great. Early in the nineteenth century Bonnemain and Jouard referred to the large number of monsters produced, and in 1809 Paris wrote, "During the period that I was at College, the late Sir Busick Harwood, the ingenious Professor of Anatomy in the University of Cambridge, frequently attempted to develope eggs by the heat of his hotbed, but he only raised monsters, a result which he attributed to the unsteady application of the heat".

PLATE VIII

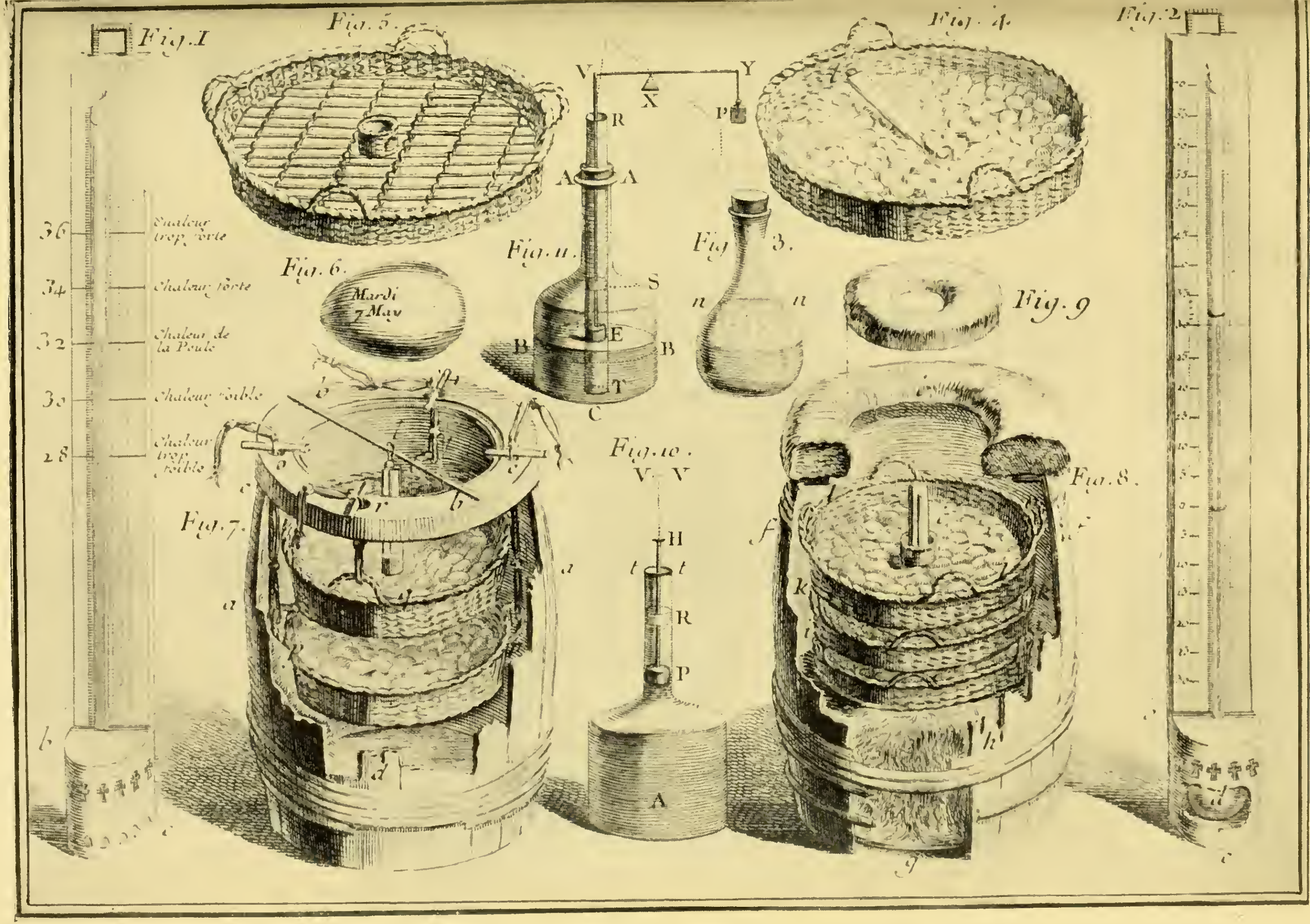


Fig. 1
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Mardi 7 May
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Fig. 8.
Fig. 9
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Fig. 11.
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Chaleur trop forte
Chaleur forte
Chaleur de la Poule
Chaleur foible
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RÉAUMUR'S ILLUSTRATION OF HIS INCUBATORS.
From *De l'art de faire éclore les poulets*, 1749.

This is the most convenient place to mention theological embryology once again. Its place in the eighteenth century was small, and in the nineteenth, with the recognition that whatever the soul is, it is not a phenomenon, it altogether disappeared from serious general discussion. F. E. Cangiamilla's *Embryologia Sacra*, however, ran through several editions between 1700 and 1775. Cangiamilla (*Panorm. Eccl. Can. Theol. et in toto Sicil. regno contra haereticam pravitatem Inquisitore provinciali*) deals very fully with the time of animation, quoting a host of writers such as St Gelasius, St Anselm, Hugh of St Victor and Pico della Mirandola. His mind retains a quite mediaeval conformation, as the following curious passage illustrates: "*Quot non foetus abortivos ex ignorantia obstetricum et matrum excipit latrina, quorum anima, si Baptismate non fraudaretur, Deum in aeternam videret, esset decentius tumulandum!*" His instructions for the baptism of monsters are also very odd. But theological embryology probably reached its climax in the report of the Doctors of Divinity at the Sorbonne on March 30, 1733, in which intra-uterine baptism by means of a syringe was solemnly recommended. This is included in Deventer's book, and has been referred to by Sterne and Spencer. For other aspects of these tracts of thought see Nicholls and his anonymous antagonist. But Cangiamilla and his colleagues—Gerike, Kaltschmied, etc.—are only of decorative importance to our present theme, and for fuller information regarding them, reference must be made to the treatise of Witovski. It is interesting to note that as late as 1913, 182 days was fixed as "perfection-time", whatever that may be, by Moriani.

3·12. Ovism and Animalculism

We must now return to the beginning of the century in order to pick up the thread of the main trend of thought. By 1720 the theory of preformation was thoroughly established, not only on the erroneous grounds put forward by Malpighi and Swammerdam, but on the experiments of Andry, Hartsoeker, Dalenpatius and Gautier, who all asserted that they had seen exceedingly minute forms of men, with arms, heads, and legs complete, inside the spermatozoa under the microscope. Gautier went so far as to say that he had seen a microscopic horse in the semen of a horse (he gave a plate of it) and a similar animalcule with very large ears in the semen of a donkey; finally, he described minute cocks in the semen of a cock.

Haller remarks gently that he has searched for these phenomena in vain. Vallisneri asserted the same kind of thing about the mammalian ovum, though he admitted that, in spite of long searching, he had never seen one. Besides the main distinction between preformationists and epigenesists, then, there arose a division among the former group, so that the ovists regarded all embryos as being produced from smaller embryos in the unfertilised eggs, while the animalculists regarded all embryos as being produced from the smaller embryos provided by the male in his spermatozoa. The animalculists thus afforded a singular example of a return to the ancient theory mentioned by Aeschylus in the *Oresteia* (see p. 65). Their most conspicuous example was Nicholas Andry, who pictured each egg as being arranged like the Cavorite sphere in which H. G. Wells' explorers made their way to the moon, i.e. with one trap-door. The spermatozoa, like so many minute men, all tried to occupy an egg, but as there were far fewer eggs than spermatozoa, there were, when all was over, only a few happy animalcules who had been lucky enough to find an empty egg, climb in, and lock the door behind them.

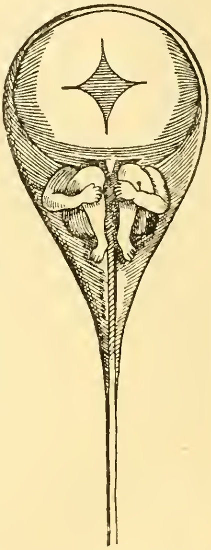


Fig. 11. Hartsoeker's drawing of a human spermatozoon.

The whole controversy was intimately bound up with the question of spontaneous generation, for, whatever the case might be in the higher animals, if it were true that the lower ones could arise *de novo* out of slime, mud, or meat infusion, for instance, then their parts at least must have been made by epigenesis, and not in any other way, for it could hardly be held that a homogeneous infusion had any structure of that kind. And if epigenesis could occur in the lower animals, then the thin end of the wedge had been driven in, and it might occur among the higher ones as well. It was in this way that the spontaneous generation controversy came to have a peculiar importance for embryology in the eighteenth century. Driesch has essayed to make the generalisation that all the supporters of epigenesis were vitalistic in their tendencies, while those who adhered to the preformation theory were not. But there are too many exceptions to this rule to make it of any use. In so far as there is truth in it, it doubtless arose from the fact that, in epigenesis, a continual production of new organs and new relationships between organs already formed would seem to require an immanent formative force of some kind, such as the *vis essentialis* of Wolff; while, on the preformation hypothesis, where embryogeny was little more than a swelling up of parts already there, it could be explained as simply as nutrition. The failure of the "short-cut" mechanical philosophers such as Gassendi and Descartes thus led to preformationism just as much as to epigenesis. A remark of Cheyne's throws much light on this question, for in 1715 he wrote, unconsciously following Gassendi's line of thought, "If animals and vegetables cannot be produced from matter and motion (and I have clearly proved that they cannot), they must of necessity have existed from all eternity". Preformationism was thus the only resource if the universal jurisdiction of the mechanical theory of the world was to be retained. Stahl and, later, Wolff, saw no point in retaining it, and carefully joined together what Descartes had, with equal care, put asunder.

The original discoveries of de Graaf and Stensen were extended by Tauvry in 1690 to the tortoise, and by Lorenzini in 1678 to the *Torpedo*: so that the eighteenth century began with an excellent basis for ovistic preformationism. The greatest names associated with this school were Swammerdam, Malpighi, Bonnet, v. Haller, Winslow, Vallisneri, Ruysch and Spallanzani. But there were many others, some of whom did valuable work, such as Bianchi, Sterre, Teichmeyer, Weygand, Perrault, Vercelloni, Vidussi, Bussière, Fizes and Coschwitz. The treatises of Imbert and Plonquet were written from this point of view, as was the bright little dialogue of de Houpeville. J. B. du Hamel asserted that he could see the chick embryo in the egg before fertilisation, and Jacobaeus made a like affirmation in the case of the frog.

On the other side, that of animalculistic preformationism, the contestants were fewer. Their greatest names were Leeuwenhoek, Hartsoeker, Leibnitz and the cardinal de Polignac. In England the physicians Keil and Cheyne supported this position, in France Geofroi and the obstetrician la Motte, in Germany Withof and Ludwig, and in Belgium Lieutaud. De Superville wrote in favour of it in the *Philosophical Transactions of the Royal Society*, and an anonymous Swedish work of some fame supported it. To the argument of Vallisneri that the existence of so many animalcules must be an illusion, since Nature could hardly be so prodigal, the animalculists retorted by instancing such observations as that of Baster, who had taken the trouble to count the eggs of a crab and had found that they amounted to 12,444. James Cooke later elaborated a theory of a world of the unborn to which the spermatozoa could retire between each attempt to find a uterus in which they could develop—this avoided Vallisneri's argument. "All those other attending Animalcula, except that single one that is then conceived, evaporate away, and return back into the Atmosphere again, whence it is very likely they immediately proceeded; into the open Air, I say, the common Receptacle of all such disengaged minute sublunary bodies; and do there circulate about with other Semina, where, perhaps, they do not absolutely die, but live a latent life, in an insensible or dormant state, like Swallows in Winter, lying quite still like a stopped Watch when let down, till they are received afresh into some other Male body of the proper kind, to be again set on Motion, and ejected again in Coition as before, to run a fresh chance for a lucky Conception; for it is very hard to conceive that Nature is so idly luxurious of Seeds thus only to destroy them, and to make Myriads of them subservient to but a single one." But Cooke's attractive hypothesis, published in 1762, came too late, as Punnett says, to save the animalculists.

On the experimental side, Garden and Bourguet came forward with descriptions of little men inside the animalcules, thus confirming the work of Gautier and Hartsoeker. It is fair to add, however, that Garden held quite enlightened views of the mutual necessity of egg and spermatozoon. La Motte maintained that the egg (which he identified with the Graafian follicle) was too big to go down the Fallopian tube, and Sbaragli, another writer on the animalculist side, agreed with him.

Leeuwenhoek, it must be admitted, indulged in assertions no less fantastic than those of his followers. He said there were spermatic animalcules of both sexes, as one could see by a slight difference near their tails, that they copulated, that the females became pregnant and gave birth to little animalcules, that young and feeble ones could be seen, that they shed their skins, and, finally, that some had been observed with two heads. Haller, who made good use, on the whole, of his strong vein of scepticism, characterised all these remarks as "only conjectures". (See Fig. 12.)

As for the supporters of epigenesis, they were few, but they included Descartes, de Maupertuis, Antoine Maître-Jan and John Turberville Needham. Von Haller affords some evidence against the identification of epigenesis with vitalism and preformation with mechanism, for he says, "Various authors have taught that the parts of the human body are formed by a mechanism depending on general laws (i.e. laws not simply of biological jurisdiction) or by the virtue of some ferment, or by rest and cold making crusts out of the different juices, or in other ways. All these (mechanical) systems have some resemblance to that of M. Wolff". Haller also speaks always of Wolff's *vis essentialis* as "blind". Minor writers on the epigenetic side were Tauvry, Welsh, Dartiguelongue, Bouger, Drelincurtius and Mazin. After 1750 C. F. Wolff brought an abiding victory to their opinion.

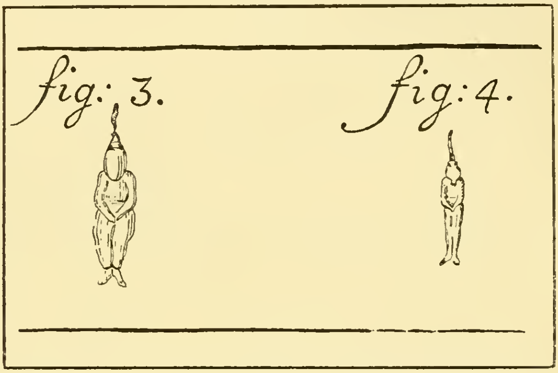


Fig. 12. Dalenpatius' drawings of human spermatozoa.

Some maintained a quite independent position, such as Buffon, who welded together an epigenetic theory of fertilisation with a preformationist theory of embryogeny. Pascal (not the great Jansenist) put forward the chemical view that fertilisation consisted of a combination between the acid semen of the male and the "lixivious" semen of the female, no doubt because in chemistry acids were regarded as male and alkalies female. Claude Perrault and Connor also suggested that the formation of the embryo was a fermentation set up in the egg by the spermatic animalcule. In this they were following the example of van Helmont, who had originally suggested such a theory. In 1763 Jacobi discovered how to fertilise fish eggs with milt; a practical matter which had a good deal of influence on biological theory. Launai alone still held to the Aristotelian conception of form and matter.

There is no need here to do more than glance at the spontaneous generation controversy itself, for it has always been well known in the history of biology, especially in connection with the subsequent work of Pasteur. J. T. Needham's books, *New Microscopical Discoveries* of 1745 and *Observations upon the generation, composition, and decomposition of animal and vegetable substances* of 1750, exercised a considerable influence. They were written after the French fashion (Needham had been educated at Douai) very concisely, and with some brilliance of style, and it is hardly true to say, as Ràdl does, that their experimental foundation was meagre. That it was inadequate was proved definitely as events turned out by Spallanzani. De Kruif's account of the controversy is false and misleading, especially in its estimate of Needham who is much more truly described in the words of Louis Pasteur (see also Prescott).

Needham's case rested upon the statement that, if meat broth was placed in a sealed vessel and heated to a high temperature so that all life was destroyed in it, it would yet be found to be swarming some days later with microscopical animals. All depended, therefore, upon the sureness with which the vessel had been sealed and the efficacy of the heat employed to kill all the animalcules initially present, and, in the ensuing controversy, Needham lost to Spallanzani entirely on a question of technique. It may be remarked here, without irrelevance, that the problem is still unsolved; for all that was proved by the experiments of Spallanzani was that animals the size of rotifers and protozoa do not originate spontaneously from broth, and all that was proved by those of Pasteur was that organisms the size of bacteria do not originate *de novo* in that way. The knowledge which we have acquired in recent years of filter-passing organisms, such as the mosaic disease of the tobacco-plant, and phenomena such as the bacteriophage of Twort and d'Herelle, has reopened the whole matter, so that of the region between, for example, the semi-living particles of the bacteriophage (10^{-15} gram) and the larger sized colloidal aggregates (10^{-18} gram) we know absolutely nothing. The dogmatism with which the biologists of the early twentieth century asserted the statement *omne vivum ex vivo* was therefore, like most dogmatisms, ill-timed.

But to dwell further on this would be a digression. The important point was that Spallanzani's victory was a victory not only for those who disbelieved in spontaneous generation, but also for those who believed in the preformation theory of embryogeny. By 1786, indeed, that viewpoint was so orthodox that Sénébier, in his introduction to an edition of Spallanzani's book on the generation of animals and plants, could treat the epigenesists as no better than atheists.

Spallanzani's views on embryology were largely drawn from his study of the development of the frog's egg. Here he went far beyond Bosius, but, in spite of many careful observations, he thought he saw the embryo already present in the unfertilised ova. This led him to claim that amphibia ought to be numbered among viviparous animals. His principal step forward was his recognition of the semen as the actual agent in fertilisation on definite experimental grounds—the narrative of his artificial insemination of a bitch is too famous to quote; he said it gave him more intellectual satisfaction than any other experiment he had ever done. This demonstration finally disposed of the *aura seminalis* which Harvey had found himself obliged to adopt on the grounds of his dissections on does. Curiously enough Spallanzani never convinced himself that the spermatozoa themselves were the active agents.

3·13. Preformation and Epigenesis

Of all the preformationists Charles Bonnet was the most theoretical. He was an adherent of that way of thinking mainly on the theoretical ground that the organs of the body were linked together in so intimate a manner that it was not possible to suppose there could ever be a moment when one or two of them were absent from the ranks. "One needs", he said, "no Morgagni, no Haller, no Albinus to see that all the constituent parts of the body are so directly, so variously, so manifoldly, intertwined as regards their functions, that their relationship is so tight and so indivisible, that they must have originated all together at one and the same time. The artery implies the vein, their operation implies the nerves which in their turn imply the brain and that by consequence the heart, and every single condition a whole row of other conditions." Bonnet compared epigenesis to crystal-growth in which particles are added to the original mass independently of the plan or scheme of the whole, i.e. in opposition to the growth of an organism, in which particles are added on only at certain places and certain times under the guidance of "forces de rapport". Przibram has recently discussed the question of how far such a comparison is admissible, but, in Bonnet's time at any rate, it became very famous. Bonnet made reference to Haller's discovery of the intimate relationship between embryo and yolk as evidence for his theory. The embryo begins, according to him, as an exceedingly fine net on the surface of the yolk, fertilisation makes part of it beat and this becomes the heart, which, sending blood into all the vessels, expands the net. The net or web catches the food particles in its pores, and Bonnet supposed that, if it were possible to abstract all the food particles at one operation from the adult animal, it would shrivel and shrink up into the original invisible web from which it originated.

Bonnet was no more afraid of the *emboîtement* principle than was Haller; indeed, he called it "one of the greatest triumphs of rational over sensual conviction". Many of his arguments were reproductions of Haller's, and he says in his preface that he had written his book some time before Haller's papers on the chick appeared, but then, finding his own views confirmed by the more experimentally founded ones of Haller, he determined to publish what he had set down. Thus in one place he says, "I shall be told, no doubt, that the observations on the development of the chick in the egg and the doe in the maternal uterus make it appear that the parts of an organised body are formed one after another. In the chick for instance it has been observed that during the early part of incubation the heart seems to be outside the animal and has a very different form to what it will have. But the feebleness of this objection is easy to apprehend. Some people wish to judge of the time when the parts of an organised body begin to exist by the time when they become visible to us. They do not reflect that minuteness and transparency alone can make these parts invisible to us although they really exist all the time".

Bonnet was therefore what might be called an "organicistic preformationist", for his objection to epigenesis lay in the fact that it apparently did not allow for the integration of the organism as a whole. His mistake was that he assumed the capacities of the adult organism to be present all through foetal life, whereas the truth is that they grow and differentiate in exactly the same way as the physical structure itself does. Bonnet's philosophical position, which has been analysed by Whitman, seriously contradicts the generalisation of Driesch that all the epigenesists were vitalists and all the preformationists mechanists. For Bonnet an epigenetic and a mechanical theory were one and the same; he hardly distinguished, as Ràdl says, between Descartes and Harvey; and it was just the neo-vitalistic idea of the organism as a whole that he could not fit in with epigenesis. Needham and Wolff were undoubtedly epigenesist-vitalists, and Bonnet was undoubtedly a preformationist-vitalist, but Maupertuis was equally clearly an epigenesist-mechanist.

G. L. Leclerc, Comte de Buffon, the most independent figure in the controversy, stood alone as much because of his erroneous experiments as because of his originality of mind. As has so often been observed, Buffon was not really an experimentalist at all: he was a writer, and preferred other people to do his experiments for him. The volume on generation in his *Histoire Naturelle* begins with a very long historical account of the work that had been done in the previous centuries on embryology. At the beginning of the section on reproduction in general he said, "The first and most simple manner of reproduction is to assemble in one body an infinite number of similar organic bodies and to compose the substance in such a manner that every part shall contain a germ or embryo of the same species and which might become a whole of the same kind with that of which it constitutes a part". Such an idea resembles the ancient atomistic speculations, and is explicated by W. Smellie, the obstetrician, who translated Buffon into English, as follows: "The intelligent reader will perceive that this sentence, though not very obvious, contains the principle upon which the whole theory of generation adopted by the author is founded. It means no more than that the bodies of animals and of vegetables are composed of an infinite number of organic particles, perfectly similar, both in figure and substance, to the whole animal or plant of which they are the constituent parts". This conception explains Buffon's curious attitude to the preformation question. An embryo was preformed in its germ because all the parts of the germ were each a model of the animal as a whole, but it was also formed by epigenesis because, the sexual organs being first formed, all the rest arose entirely by a succession of new origins. Buffon's "organic, living, particles" bear some resemblance to the "biogen molecules" which later generations were to discuss, and he says that an exactly similar but simpler structure is present in dead matter.

In his discussion of former theories he resolutely rejects the *emboîtement* aspect of preformationism, giving various calculations to show its impossibility and maintaining that "every hypothesis which admits an infinite progression ought to be rejected not only as false but as destitute of every vestige of probability. As both the vermicular and ovular systems suppose such a progression, they should be excluded for ever from philosophy". He completely destroys the theory which the ovists and animalculists had set up in order to explain resemblance to parents, namely, that, although the foetus might originate either from egg or spermatic animalcule originally, it was moulded into the form of its parents by the influence of the maternal organism during pregnancy. This field, which was more than once disturbed by the contestants during the course of the century, received systematic attention from time to time by medical writers. There was a memorable dispute on this point between Turner and Blondel, whose polemics, written in an exceedingly witty manner, are still very pleasant and amusing to read. Blondel was the sceptic and Turner the defender of the numerous extraordinary stories which passed for evidence on this subject. It is interesting to note that Turner believed in the continuity of foetal and maternal blood-vessels. Krause and Ens later supported the opinions of Turner, while Okes, in a Cambridge disputation, argued against them.

Buffon's sixth chapter, in which he relates the progress of his own experiments, is unfortunate, in that his main result was to discover spermatozoa in the *liquor folliculi* of ovaries of female animals. The explanation of how he came to make such an enormous mistake has never been satisfactorily given, and it was not long before the truth of the observation was questioned by Ledermuller. It led him naturally to the assertion that the ovaries of mammalia were not egg-producing organs but animalcule-producing organs, and to the view that the beginning of embryonic development lay in the fusion of the male with the female spermatic animalcules—a curious revival of Epicureanism. But it is to be observed that he does not mean one male animalcule with one female animalcule, but rather all with all, in a kind of pangenesis. "All the organic particles", he says, "which were detached from the head of the animal will arrange themselves in a similar order in the head of the foetus. Those which proceeded from the backbone will dispose themselves in an order corresponding to the structure and position of the vertebrae". And so on for all the organs. The fact that for the organs common to both sexes a double set of animalcules will thus be provided does not give Buffon any difficulty and is fully admitted by him. Accordingly he could only agree to the aphorism *omne vivum ex ovo* in the sense of

Harvey, namely, as referring to the egg-shaped chorion of vivipara, and definitely not in the sense of de Graaf and Stensen, namely, in the modern sense. "Eggs", he says, "instead of being common to all females, are only instruments employed by Nature for supplying the place of uteri in those animals which are deprived of this organ. Instead of being active and essential to the first impregnation, eggs are only passive and accidental parts, destined for the nourishment of the foetus already formed in a particular part of this matrix by the mixture of the male and female semen." Biology at this period was still labouring under the disadvantage of being without the cell-theory, and therefore unable to distinguish between an egg and an egg-cell.

In spite of his leanings towards epigenesis, Buffon repeats precisely the error of Malpighi. "I formerly detected", he says, "the errors of those who maintained that the heart or the blood was first formed. The whole is formed at the same time. We learn from actual observation that the chicken exists in the egg before incubation. The head, the backbone, and even the appendages which form the placenta are all distinguishable. I have opened a great number of eggs both before and after incubation and I am convinced from the evidence of my own eyes that the whole chicken exists in the middle of the cicatrice the moment the egg issues from the body of the hen. The heat communicated to it by incubation expands the parts only. But we have never been able to determine with certainty what parts of the foetus are first fixed, at the moment of its formation." The experiment of taking a look at the cicatrices of eggs on their way down the parental oviduct is so obvious that Buffon must have thought of it, and it would be really interesting to know what factor in the intellectual climate it was that made him regard such an observation as not worth attempting. His observations on the embryo itself were good and, in some ways, new; thus he noticed that the blood first appears on the "placenta" or blastoderm, and for the first few days seems hardly to enter the body of the embryo. He gave an extremely good account of the whole developmental process in the chick and in man, and his opinions on the use of the amniotic liquid and the functions of the umbilical cord were very advanced.

J. T. Needham, however, spoke very clearly in favour of epigenesis, though he himself did no embryological experiments. His *Idée sommaire* of 1776, written against Voltaire, who had called him a Jesuit and who had drawn materialistic inferences from his writings, contained the following passage: "The numerous absurdities which exist in the opinion of pre-existent germs together with the impossibility of explaining on that ground the birth of monsters and hybrids, made me embrace the ancient system of epigenesis, which is that of Aristotle, Hippocrates, and all the ancient philosophers, as well as of Bacon and a great number of savants among the neoteriques. My observations also led me directly to the same result". Needham's embryology is mostly contained in his *Observations nouvelles sur la Génération* of 1750. He was explicitly a Leibnitzian and postulated a vegetative force in every monad.

Needham was not the only thoroughgoing epigenesist of this period. Maupertuis, whose *Vénus Physique* was published anonymously in 1746, came out very clearly on the side of epigenesis. "I know too well", he said, "the faults of all the systems which I have been describing, to adopt any one of them, and I find too much obscurity in the whole matter to wish to form one of my own. I have but a few vague thoughts which I propose rather as thoughts to be examined than as opinions to be received, and I shall neither be surprised nor think myself aggrieved if they are rejected. It seems to me that both the system of eggs and that of spermatic animalcules are incompatible with the manner in which Harvey actually saw the embryo to be formed. And one or the other of these systems seems to me still more surely destroyed by the resemblance of the child, now to the father and now to the mother, and by hybrid animals which are born from two different species....In this obscurity in which we find ourselves on the manner in which the foetus is formed from the mixture of two liquors, we find certain facts which are perhaps a better analogy than what happens in the brain. When one mixes silver and spirits of nitre with mercury and water, the particles of these substances come together themselves to form a vegetation so like a tree that it has been impossible to refuse it the name." This was the *Arbor Dianae*, which played a great part in these embryological controversies of the eighteenth century. It has a great interest for us, for it was perhaps the first occasion on which a non-living phenomenon had been appealed to as an illustration of what went on in the living body. It is true that Descartes long before had said that the movements of the living body were carried out by mechanisms like clocks or watches, and that they resembled the statues in certain gardens which could be made to perform unexpected functions by the pressure of a manipulator's foot on a pedal, but these instances were all artificially constructed mechanical devices, whereas the *Arbor Dianae* was a natural phenomenon quite unexplained by the chemists of the time, and the lineal forerunner of Lillie's artificial nerve, and Rhumbler's drop of chloroform. We know now that its formation is a simpler process than anything which occurs in the developing embryo, but the course of research has made it undeniably clear that the same forces which operate in the formation of the *Arbor Dianae* are at work also in the developing embryo. To this extent Maupertuis is abundantly justified, and Driesch's comments on him are not in agreement with the facts.

"Doubtless many other productions of a like kind will be found", Maupertuis goes on, "if they are looked for or perhaps if they are looked for less. And although they seem to be less organised than the body of most animals, may they not depend on the same mechanics and on similar laws? Will the ordinary laws of motion suffice, or must we have recourse to new forces? These forces, incomprehensible as they are, appear to have penetrated even into the Academy of Sciences at Paris, that institution where so many opinions are weighed and so few admitted." Maupertuis goes on to speak of the contemporary deliberations on the subject of attraction. "Chymistry", he says, "has felt the necessity of adopting this conception and attractive force is nowadays admitted by the most famous chymists who have carried the use of it far beyond the point which the astronomers had reached. If this force exists in nature, why should it not take part in the formation of animals?" Maupertuis was thus an epigenesist and a mechanist at the same time. His opinions have an extremely modern ring, and his only retrograde step was in suggesting that the spermatic animals had nothing else to do except to mix the two seeds by swimming about in them. But that legacy of ovism was common all through the eighteenth century, and thirty years later Alexander Hamilton could say, "From the discovery of Animalcula in *semine masculino* by Leeuwenhock's Glasses, a new Theory was adopted which is not yet entirely exploded".

But the real middle point and fulcrum of the whole period lay in the controversy between von Haller and Caspar Friedrich Wolff, the former at Göttingen and the latter at St Petersburg in the Academy of the Empress Catherine. Kirchhoff has described this polemic.

Wolff's *Theoria generationis*, which was a defence of epigenesis on theoretical and philosophical grounds, written in a very formal, logical, and unreadable manner, appeared when he was only twenty-six years old, in 1759. Leibnitz, as Ràdl points out, had borrowed from the earlier preformationists the conception of a unit increasing in bulk in order to become another kind of unit; but Wolff, following Needham, borrowed from Leibnitz the idea of a monad developing into an organism by means of its own inherent force, and to this he joined the Stahlian notion of a generative supra-physical force in nature. On the practical side, Wolff's work was indeed of the highest importance. If the embryo pre-exists, he argued, if all the organs are actually present at the very earliest stages and only invisible to us even with the highest powers of our microscopes, then we ought to see them fully formed, as soon as we see them at all. In other words, at the moment at which any given organ comes into view, it ought to have the form and shape, though not the size, of the same organ when fully completed in the embryo at birth. On the other hand, if this is not the way in which development goes on, then one ought to be able to see with the microscope one shape changing into another shape, and, in fact, a series of appearances, each one different from that which had immediately preceded it, or, in other words, a series of advancing adaptations of the various parts of the primitive embryonic mass. Wolff chose as his first test case the blood-vessels of the blastoderm in the chick, for he saw that at one moment this apparatus was in existence, while the moment before it had not been. His microscopical researches led him to the conclusion that the homogeneous surface of the blastoderm partially liquefies and transforms itself at these points into a mass of islands of solid matter, separated by empty spaces filled with a colourless liquid but afterwards with a red liquid, the blood. Finally, these spaces are covered with membranes and become vessels. Consequently it was obvious that the vessels had not been previously formed, but had arisen by epigenesis.

Haller replied to this new experimental foundation for epigenesis without delay, for he was working on the development of the chick at the same time, and held closely to the opposite theory. We have already seen what his one and only argument against Wolff was. He used it time after time in all its possible variations, maintaining stoutly that the chick embryo was so fluid in the early stages that Wolff had no right to deny the presence of a given structure simply because he could not see it. Haller's explanation of Wolff's results was that the blood-vessels had been there all the time but that they had not become visible until the moment at which Wolff saw the islands forming. "After I had written the above", said Haller, "M. Wolff made new objections against the demonstration. Instructed by new researches, he denies absolutely that the yolk-membranes, which he makes two in number, exist before incubation. He pretends that they are new and that they are born at the beginning of incubation, and consequently that the continuity of their vessels with the embryo does not in the least prove that in the body of the mother the yolk received vessels from the foetus. I have compared the observations of this great man with my own and I have found that the yolk never has more than one pulpy and soft membrane, part of which is what I have called the umbilical area, and that the fine exterior membrane does not belong to the yolk but to the inner part of the umbilical membrane....I do not believe that any new vessels arise at all, but that the blood which enters them makes them more obvious because of the colour which it gives them, and so by the augmentation of their volume, they become longer."

Wolff replied by another extensive piece of work, which he called *De Formatione Intestinorum*, and which appeared in one of the publications of the Russian Academy for 1768. It ruined preformationism. In it he demonstrated that the intestine is formed in the chick by the folding back of a sheet of tissue which is detached from the ventral surface of the embryo, and that the folds produce a gutter which in course of time transforms itself into a closed tube. The intestine, therefore, could not possibly be said to be preformed, and from this as starting-point, Wolff went on to propose an epigenetic theory which applied the same process to all organs. It is interesting to note that the facts brought forward by Wolff have never been contradicted, but have been used as a foundation to which numberless morphological embryologists have added facts discovered by themselves. It is noteworthy that, although Wolff's second general principle, that of increasing solidification during embryonic development, led to no immediate results, it has been abundantly confirmed since then (see Fig. 221). His observations on the derivation of the parts of the early embryo from "leaf-like" layers were even more important, and acted as a very potent influence in the work of Pander and von Baer.

It happened, however, that Haller had much the greater influence in the biological world at the time, so that Wolff's conceptions did not immediately yield fruit in any general advance. Looking back over the second half of the seventeenth and the first two-thirds of the eighteenth century, it is remarkable how little theoretical progress was made in view of the abundance of new facts which were discovered. Punnett, in an interesting paper, has vividly brought this out. "The controversy between the Ovists and Animalculists had lasted just a century", he says, "and it is not uninteresting to reflect that the general attitude of science towards the problem of generation was in 1775 much what it had been in 1675. When the period opened, almost all students of biology and medicine were Preformationists and Ovists; at its close they were for the most part Ovists and Preformationists." Ovism sprang in the first instance from de Graaf's discovery of the mammalian egg, which gave a new and precise meaning to Harvey's aphorism. Preformationism, already old as a theory, acquired an apparent factual basis in the work of Malpighi and Swammerdam, and allied itself naturally with ovism. With Leeuwenhoek and his spermatozoa, animalculism came upon the field. The main outlines of the battle which went on between the two viewpoints have already been drawn, but it is worth remembering that there were independent minds who were impressed by the obvious facts of heredity and found it difficult to call one sex essential rather than the other. Among these Needham and Maupertuis might be counted, and among the lesser men, James Handley with his *Mechanical Essays on the Animal Oeconomy* of 1730 ought to receive a mention. Though fond of theological arguments he upheld the common-sense attitude against ovists and animalculists alike—"We dissent in some things", he said, "both from Leeuwenhoeck and Harvey....Both the semen and ova (notwithstanding all that can be said) we believe to be a *causa sine qua non* in every Generation". But what finally killed animalculism was the discovery in so many places of small motile living beings, flagellates, protozoa, large vibrios. It was difficult to maintain in the face of this new evidence that the spermatozoa were essential elements in generation, though the seminal fluid itself might very well be, as of course was Spallanzani's opinion. The preformation theory was what was holding up further progress, and when Wolff's arguments prevailed in the very last years of the eighteenth century, the way was open for the recognition of the true value of the spermatozoa.

The otherwise unknown physician d'Aumont, who wrote the article on "Generation" in Diderot's famous *Encyclopaedia*, brought this out in an interesting way, for himself an ovist, he summarised the arguments, which, in 1757, were destroying the animalculist position, and reducing rapidly the number of its adherents.

1. Nature would never be so prolific as to produce such millions of spermatic animalcules, each one with its soul, unnecessarily.
2. The spermatic animalcules of all animals are the same size, no matter how large the animal is: how, therefore, can they be involved in its generation?
3. They are never found in the uterus after coitus, but only in the sperm (?).
4. How do they reproduce their kind?
5. What evidence is there that they are any different from the animalcules (of similar shape, etc.) which are to be found in hay infusion, scrapings from the teeth, etc.? Nobody supposes that these have any relation to reproduction.

3·14. The Close of the Eighteenth Century

The last forty years of the century were not marked by any great movement in a fruitful direction for morphological embryology, an iconographic wave of some merit due to Albinus, W. Hunter, Tarin, Senff, Rosenmuller, Danz and Soemmering excepted; and it was not until 1812 that J. F. Meckel the younger translated Wolff's papers into German. This was one of the principal influences upon Pander and von Baer. In his introduction, Meckel describes how Wolff's work had been disregarded, and points out that Oken, writing in 1806, had apparently never even heard of it. In the very early years of the nineteenth century morphological embryology received a great impetus, however. One of the most interesting figures of the new period was de Lézérec, a Breton, whose father had been in the Russian naval service. The son, as a Russian naval cadet, no doubt stimulated by the writings of Wolff, who had lived at St Petersburg, used to incubate eggs on board ship. He eventually left the sea, studied medicine at Jena, and wrote an excellent dissertation on the embryology of the chick in 1808, which Stieda has recently brought to light. He then went to Paris, and, taking a medical appointment at Guadeloupe, was lost to science. Very much more important was the work of Pander in 1817 and von Baer in 1828, but it belongs to the present period, and I shall not treat it historically. For data on von Baer, see Kirste, Addison and Stieda. It is interesting to note, however, that the recapitulation theory, which was first clearly formulated by von Baer, was already taking shape in various minds during the closing years of the eighteenth century. Lewes has thus described the thesis of Goethe's *Morphologie*, written in 1795: "The more imperfect a being is the more do its individual parts resemble each other and the more do these parts resemble the whole. The more perfect a being is the more dissimilar are its parts. In the former case the parts are more or less a repetition of the whole, in the latter case they are totally unlike the whole. The more the parts resemble each other the less subordination is there of one to the other: and subordination is the mark of high grade of organisation".

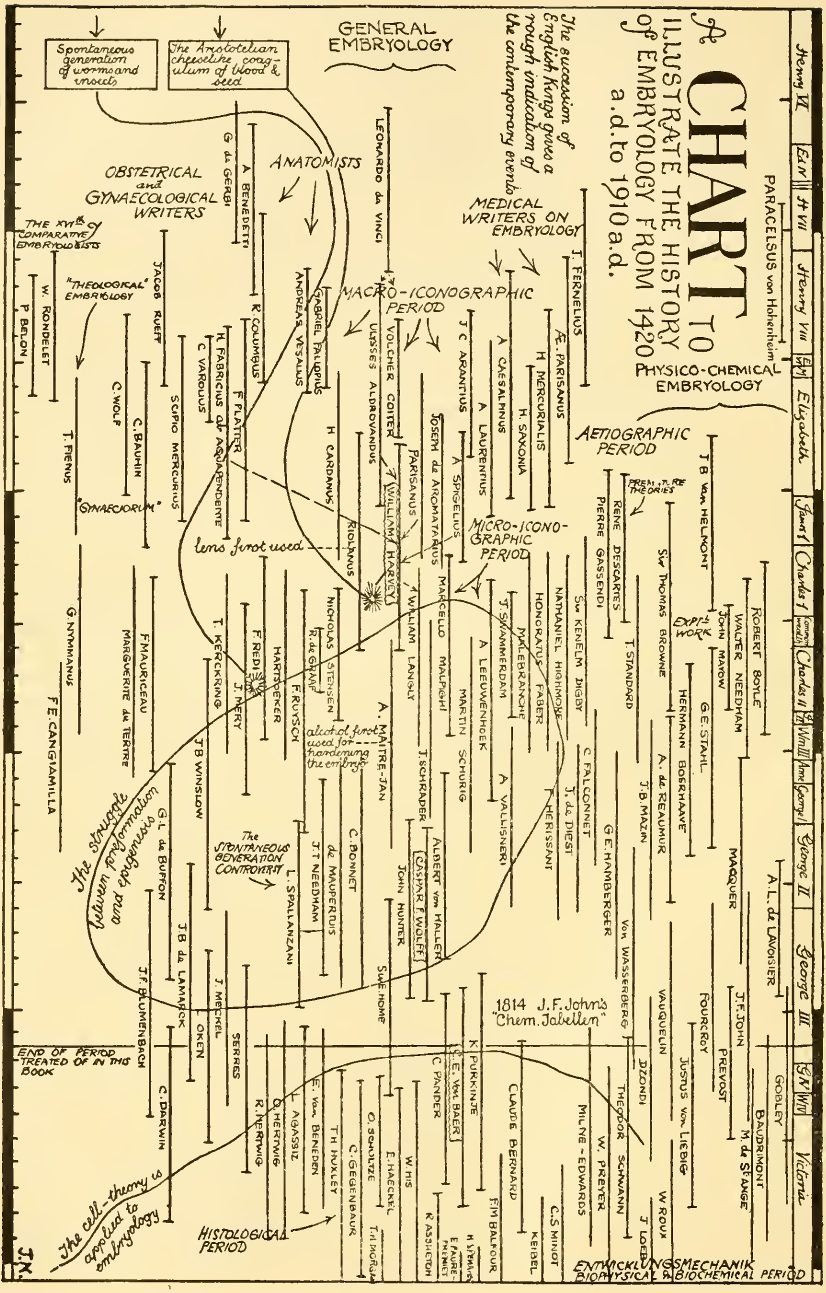
William and John Hunter belong also to the end of the century. The former, in his book on the anatomy of the gravid uterus, proved finally and completely the truth of the view that the maternal and foetal circulations are distinct. His injections left no shadow of doubt about the matter, and the way was clearly opened up for the study of the properties of the capillary endothelial membranes separating the bloods, a study which is still vigorously proceeding, especially in its physico-chemical aspect (see Section 21). There was a quarrel between the brothers over the priority of this demonstration. John Hunter's *Essays and Observations* also contain material important for embryology. His drawings of the chick in the egg were very beautiful, and are still in the archives of the Royal College of Surgeons. He adopted Mayow's theory of the office of the air-space, and anticipated von Baer's theory of recapitulation much as did Goethe. "If we were capable of following the progress of increase of the number of parts of the most perfect animal as they were first formed in succession, from the very first to its state of full perfection, we should probably be able to compare it with some one of the incomplete animals themselves, of every order of animals in the creation, being at no stage different from some of the inferior orders. Or, in other words, if we were to take a series of animals, from the more imperfect to the perfect, we should probably find an imperfect animal corresponding with some stage of the most perfect." It is impossible not to reflect on the curious course which was taken by the essence of the idea of recapitulation in the history of embryology. As Aristotle first formulated it, it was as much bodily as mental, but all his successors until the eighteenth century A.D. treated it as a psychological rather than a physiological or morphological theory, and lost themselves in speculations about the vegetative, sensitive, and rational souls. Yet the other aspect of the theory was only asleep, and was destined to be of the greatest value as soon as investigators began to direct their attention more to the material than to the spiritual aspect of the developing being.

Hunter did not absolutely reject preformationism, but regarded it as holding good for some species in the animal kingdom; he therefore attached no philosophical importance to it.

Although Wolff's work did not lead to the immediate morphological advances which might have been expected, it was in many ways fruitful. It produced J. F. Blumenbach's *Über den Bildungstrieb* of 1789, a work which elaborated the Wolffian *vis essentialis* into the *nisus formativus*, a directing morphogenetic force peculiar to living bodies. It is interesting to note that Blumenbach passed through an exactly opposite succession of opinions to that of Haller, i.e. he was first attracted by preformationism, but, being convinced by Wolff's work, abandoned it in favour of epigenesis. Blumenbach compares his *nisus formativus* with the force of gravity, regarding them as exactly similar conceptions and using them simply as definitions of a force whose constant effects are recognised in everyday experience. Blumenbach says that his *nisus formativus* differs from Wolff's *vis essentialis* because it actively does the shaping and does not merely add suitable material from time to time to a heap of material which is already engaged in shaping itself. Wolff was still alive at this time, but he did not make any comment on Blumenbach, though he might very well have said that Blumenbach had misunderstood him, and that their forces were really alike in every particular. Both Blumenbach and Wolff were mentioned by Kant in the *Critique of Judgement* where he adopted the epigenetic theory in his discussion of embryogeny.

A word must be said at this point about the opinions of the eighteenth century on foetal nutrition. At the beginning of it, there was, as has been shown, a welter of conflicting theories; and though, later on, writers on this subject were fewer, the progress made was no more rapid. In 1802 Lobstein was supporting the view (which had been defended by Boerhaave) that the amniotic liquid nourished the embryo *per os*, although Themel had shown forty years before

CHART III


A CHART TO ILLUSTRATE THE HISTORY OF EMBRYOLOGY FROM 1420 a.d. to 1910 a.d.
The succession of English Kings gives a rough indication of the contemporary events
PHYSICO-CHEMICAL EMBRYOLOGY
1450
1500
1550
1600
1650
1700
1750
1800
1850
1900
Henry VI
Edw IV
H VII
Henry VIII
Ed VI
M
Elizabeth
James I
Charles I
Commonwealth
Charles II
J II
Wm III
Anne
George I
George II
George III
G IV
W IV
Victoria
Spontaneous generation of worms and insects
The Aristotelian cheeselike coagulum of blood & seed
GENERAL EMBRYOLOGY
ANATOMISTS
OBSTETRICAL and GYNAECOLOGICAL WRITERS
THE XVIth cy COMPARATIVE EMBRYOLOGISTS
"THEOLOGICAL" EMBRYOLOGY
MEDICAL WRITERS ON EMBRYOLOGY
MACRO-ICONOGRAPHIC PERIOD
MICRO-ICONOGRAPHIC PERIOD
AETIOGRAPHIC PERIOD
PREFORMATIVE THEORIES
EXPTL WORK
lens first used
alcohol first used for hardening the embryo
The SPONTANEOUS GENERATION CONTROVERSY
The struggle between preformation and epigenesis
END OF PERIOD TREATED OF IN THIS BOOK
1814 J. F. John's "Chem. Tabellen"
The cell-theory as applied to embryology
HISTOLOGICAL PERIOD
ENTWICKLUNGSMECHANIK
BIOPHYSICAL & BIOCHEMICAL PERIOD
J.N.
P. BELON
W. RONDELET
T. FIENUS
"GYNAECIORUM"
G. NYMMANUS
F.E. CANGIAMILLA
C. WOLF
C. BAUHIN
JACOB RUEFF
SCIPIO MERCURIUS
F. MAURICEAU
MARGUERITE du TERTRE
G.L. de BUFFON
J.B. de LAMARCK
J.F. BLUMENBACH
C. DARWIN
G. da GERBI
A. BENEDETTI
R. COLUMBUS
C. VAROLIUS
H. FABRICIUS ab AQUAPENDENTE
F. PLATTER
T. KERCKRING
J.B. WINSLOW
OKEN
J. MECKEL
SERRES
F. REDI
J. MERY
HARTSOEKER
F. RUYSCH
R. de GRAAF
NICHOLAS STENSEN
L. SPALLANZANI
J.T. NEEDHAM
de MAUPERTUIS
C. BONNET
ANDREAS VESALIUS
GABRIEL FALLOPIUS
H. CARDANUS
RIOLANUS
ULISSES ALDROVANDUS
LEONARDO da VINCI
VOLCHER COITER
WILLIAM HARVEY
PARISANUS
JOSEPH de AROMATARIUS
WILLIAM LANGLY
A. MAITRE-JAN
J. SCHRADER
JOHN HUNTER
Sir E. HOME
ALBERT von HALLER
CASPAR F. WOLFF
MARCELLO MALPIGHI
MARTIN SCHURIG
J.C. ARANTIUS
A. SPIGELIUS
A. LAURENTIUS
A. CAESALPINUS
H. SAXONIA
H. MERCURIALIS
AE. PARISANUS
J. FERNELIUS
T. SWAMMERDAM
A. LEEUWENHOEK
MALEBRANCHE
HONORATUS FABER
NATHANIEL HIGHMORE
A. VALLISNERI
HERISSANT
Sir KENELM DIGBY
C. FALCONNET
J. de DIEST
PIERRE GASSENDI
RENE DESCARTES
T. STANDARD
G.E. HAMBERGER
J.B. MAZIN
Sir THOMAS BROWNE
A. de REAUMUR
von WASSERBERG
HERMANN BOERHAAVE
G.E. STAHL
JOHN MAYOW
WALTER NEEDHAM
ROBERT BOYLE
J.B. van HELMONT
PARACELSUS von Hohenheim
MACQUER
A.L. de LAVOISIER
J.F. JOHN
FOURCROY
VAUQUELIN
PREVOST
C. PANDER
K. PURKINJE
C.E. von BAER
CLAUDE BERNARD
MILNE-EDWARDS
THEODOR SCHWANN
DZONDI
JUSTUS von LIEBIG
GOBLEY
BAUDRIMONT
M. de ST. ANGE
W. PREYER
W. ROUX
J. LOEB
C.S. MINOT
KEIBEL
F.M. BALFOUR
H. SPEMANN
E. FAURE-FREMIET
R. ASSHETON
T.H. MORGAN
W. HIS
E. HAECKEL
O. SCHULTZE
C. GEGENBAUR
T.H. HUXLEY
E. van BENEDEN
L. AGASSIZ
O. HERTWIG
R. HERTWIG

that this could be at most the very slightest source of material, from a study of acephalic monsters. These workers had obviously learnt nothing from Herissant and Brady, who had been over precisely the same ground fifty years before. On the other hand, Goods and Osiander reported the birth of embryos without umbilical cords, so that the solution of this question became, in the first year of the nineteenth century, balanced, as it were, between the relative credibility of two kinds of prodigy. Nourishment *per os* was defended by Kessel, Hannes and Grambs, and was attacked by Vogel, Bernhard, Glaser, Hannhard and Reichard. The idea lingered on right into the modern period, and as late as 1886 von Ott, who was much puzzled about placental permeability, decided that a great part in foetal nutrition must be played by the amniotic liquid. Weidlich, a student of his, fed a calf on amniotic liquid for some days, and as it seemed to get on all right, he reported the amniotic liquid to have nutritive properties. The appeal to monsters was still resorted to at the end of the nineteenth century, for Opitz, in order to negative von Ott's conclusions, drew attention to a specimen in the Chemnitz Polyklinik in which the oesophagus of a well-nourished normal infant was closed at the upper third without the development of the body having been in any way restricted. The fuller possibilities of biochemistry itself have sometimes been exploited in favour of the ancient theory of nourishment *per os*; thus Köttnitz in 1889 collected some data about the presence of peptones and protein in the human amniotic liquid with this object in view. That the foetus swallows the liquid which surrounds it towards the end of gestation in all amniota, can hardly be disputed, and as there are known to be active proteolytic enzymes in the intestinal tract, no doubt some of the protein which it contains is digested—but to maintain that any significant part is played in foetal nutrition by this process has become steadily more and more impossible since 1600.

But to return to the eighteenth century; all was not repetition; occasionally somebody brought forward a few facts. Thus the deglutition of the amniotic liquid was discussed by Flemyng in 1755 in a paper under the title "Some observations proving that the foetus is in part nourished by the amniotic liquor". "I believe", he said, "that very few, if any at all, will maintain now-a-days with Claudius de la Courvée and Stalpart van-der-Wiel, that the whole of its nourishment is conveyed by the mouth." But he himself had found white hairs in the meconium of a calf embryo with a white hide. Both Aldes and Swammerdam had found the same thing, but Aldes did not think it of any significance, and Swammerdam merely remarked that the calf must lick itself *in utero*.

More interesting was W. Watson's "Some accounts of the foetus *in utero* being differently affected by the Small Pox". This was the earliest investigation of the permeability of the placenta to pathological agents. "That the foetus", said Watson, "does not always partake of the Infection from its Mother, or the Mother from the Foetus, is the subject of this paper." Two of his cases, he said, "evince that the Child before its Birth, though closely defended from the external Air, and enveloped by Fluids and Membranes of its own, is not secure from the variolous Infection, though its Mother has had the Distemper before. They demonstrate also the very great Subtility of the variolous Effluvia". But other cases "are the very reverse of the former, where though from Inoculation the most minute portion of Lint moisten'd with the variolous Matter and applied to the slightly wounded Skin, is generally sufficient to propagate this Distemper; yet here we see the whole Mass of the Mother's Blood, circulating during the Distemper through the Child, was not sufficient to produce it....From these Histories it appears that the Child before its Birth ought to be consider'd as a separate, distinct Organization; and that though wholly nourish'd by the Mother's Fluids, with regard to the Small Pox, it is liable to be affected in a very different Manner and at a very different Time from its Mother". Doubtless the modern explanation of Watson's discordant results would be that in one case there were placental lesions, destroying the perfect barrier between the circulations, and in others there were not.

In the last year of the century (but the seventh of the Republic) Citizens Léveillé & Parmentier contributed an interesting paper to the *Journal de Physique* in which they observed the increase in size of the avian yolk on incubation and spoke of a current of water yolkwards (see Fig. 225).

3·15. The Beginning of the Nineteenth Century

At the beginning of the new century a fresh influence came in with the work of Lamarck, though it did not have such a great effect on his contemporaries as on later generations. Its relations with biochemistry are so remote that there is no need to deal in any detail with it here, but Lamarck's opinions on embryology may perhaps be given in the words of Cuvier, written in 1836.

"In 1802 he published his researches on living bodies, containing a physiology peculiar to himself, in the same way that his researches on the principal facts of physics contained a chemistry of that character. In his opinion the egg contains nothing prepared for life before being fecundated, and the embryo of the chick becomes susceptible of vital motion only by the action of the seminal vapour; but, if we admit that there exists in the universe a fluid analogous to this vapour, and capable of acting upon matter placed in favourable circumstances, as in the case of the embryon, which it organises and fits for the enjoyment of life, we will then be able to form an idea of spontaneous generations. Heat alone is perhaps the agent employed by nature to produce these incipient organizations, or it may act in concert with electricity. M. de Lamarck did not believe that a bird, a horse, nor even an insect, could directly form themselves in this manner; but, in regard to the most simple living bodies, such as occupy the extremity of the scale in the different kingdoms, he perceived no difficulty; for a monad or a polypus are, in his opinion, a thousand times more easily formed than the embryo of a chick. But how do beings of a more complicated structure, such as spontaneous generation could never produce, derive their existence? Nothing, according to him, is more easy to be conceived. If the orgasm, excited by this organizing fluid, be prolonged, it will augment the consistency of the containing parts, and render them susceptible of reacting on the moving fluids which they contain, and an irritability will be produced, which will consequently be possessed of feeling. The first efforts of a being thus beginning to develope itself must tend to procure it the means of subsistence and to form for itself a nutritive organ. Hence the existence of an alimentary canal. Other wants and desires, produced by circumstances, will lead to other efforts, which will produce other organs: for, according to a hypothesis inseparable from the rest, it is not the organs, that is to say, the nature and form of the parts, which give rise to habits and faculties; but it is the latter which in process of time give birth to the organs. It is the desire and the attempt to swim that produces membranes in the feet of aquatic birds; wading in the water, and at the same time the desire to avoid getting wet, has lengthened the legs of such as frequent the sides of rivers; and it is the desire of flying that has converted the arms of all birds into wings, and their hairs and scales into feathers. In advancing these illustrations, we have used the words of our author, that we may not be suspected either of adding to his sentiments or detracting any thing from them."

If the latter part of the eighteenth century did not produce the move forward in the morphological direction which might have been expected from the work of Wolff, a remarkable amount of work was accomplished on the chemical side. This mass of work did not spring from any one source, it was not due to a great discovery on the part of one man, but rather it came about that, as the technique of chemistry itself improved, a number of otherwise undistinguished investigators, such as Dehne, Macquer and Bostock, applied physico-chemical methods to the embryo, though it is true that among the names are those of certain great chemists, such as Scheele and Fourcroy. The results of this movement were summarised in the work of J. F. John, whose *Chemische Tabellen des Tierreichs* appeared in 1814. With this date I propose to bring my historical assessment to an end. The work that was done in physico-chemical embryology after 1814 will be considered in the appropriate sections dealing with the problems of the present time; for Gobley, as an example, who gave the name to the substance still called vitellin, was working only a dozen years after the date of the publication of John's *Tabellen*.

In this translation of the Tables, I have made one alteration only. John groups together a number of data which are contained in von Haller's *Elementa Physiologiae*, and attributes them to that great man. But actually they were obtained by earlier investigators and only came to John through the medium of Haller and Fourcroy—I have therefore allotted them to their true originators.

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814

Substance or liquid investigated	Composition	Investigator	Date
Amniotic liquid (man)	It contains a substance which can be precipitated with tincture of gall, phosphate of lime and muriatic salts	—	—
,,	It is salt	Rhades	1753
,,	,,	Schrader	1674
,,	It is sweet	Vieussens	1705
,,	It coagulates on boiling	Rhades	1753
,,	,, ,,	Roederer	1750
,,	,, ,,	Barbati & Heertodt	1676

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814 (*cont.*)

Substance or liquid investigated	Composition	Investigator	Date
Amniotic liquid (man)	It is miscible with water	Longfield	1759
,,	It is coagulable by tincture of gall	Rhades	1753
,,	It is coagulable by alcohol	Spielmann	1753
,,	It is coagulable by alumina	Tauvry	1690
,,	It is coagulable by spirits of nitre	Langly	1674
,,	Free mineral alkali, water, albuminous substance, common salt	Gmelin & Ebermaier	1796
,,	Much water, very little common salt, fire-stable alkali, phosphoric acid, some earth, and oxyde of iron	van den Bosch	1792
,,	Much water, a lymphatic coagulum, common salt, salmiac, a trace of phosphate of lime	Scheele	—
,,	Sp. g. 1·005. Albuminous matter, soda, muriate of soda, phosphate of lime, the rest is water	Vauquelin & Buniva	—
Cheesy material, given off into the amniotic liquid by the body of the foetus (man)	Animal slime, and a characteristic fatty material, or rather an albuminous material tending to fat, carbonate of lime	Vauquelin & Buniva	—
Embryonic tissue-juice (man)	It contains hydrofluoric acid	Berzelius	1807
Amniotic liquid (cow)	Water, much sulphate of soda, phosphate of lime and talc, an animal substance soluble in water, insoluble in spirits of wine, and not forming a combination with tannic acid, a crystalline amniotic acid	Buniva & Vauquelin	—
Amniotic and allantoic liquids (cow)	The liquid of the allantois is very different quantitatively in the different periods of pregnancy, as also in the qualitative aspect of its composition. First it is crystalline and colourless, then it gets yellowish, and finally a dark reddish-brown. But it remains watery all the time and never has the property possessed by the amniotic liquid, of becoming at last quite slimy even to the point of showing fibres in it. During the last months the hippomanes appear in it, these are soft and yet tough. The quantity of this liquid is much greater at the end than at the beginning. Alcohol precipitates from it a very large amount of a reddish substance; sulphate of baryta, tartaric acid, and carbonate of lime give a large precipitate. These reagents do not change the amniotic fluid at all. 1000 gm. liq. allant. gave 20–25 gm. solid residue, 1000 gm. liq. amnii gave 10–11 gm. solid residue	Dzondi	1806

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814 (*cont.*)

Substance or liquid investigated	Composition	Investigator	Date
Blood of embryo (man)	Soda, much serum, and some leathery fibrous threads, which made up only $\frac{1}{2}$ grain out of 3 gros 6 grains of cruor. They were jelly-like in consistency. No phosphoric acid. It differed from the blood of an adult (1) in not giving a red flush when shaken up with air, (2) in not clotting in air, (3) in the fibres being more jelly-like	Fourcroy	1790
Blood of embryo (rabbit)	Does not coagulate in the cold but gives rise to a red serum tending towards brown. It was not as solid as usual except when heated, then it went grey though the supernatant liquor was red	Fourcroy	—
Foetal urine (man)	It is odourless and colourless and of a slimy nature	Fourcroy	1803
Meconium (man)	Water $\frac{4}{5}$, $\frac{1}{10}$ spirituous extract similar to gall, a black residue dissolving partially in water to give a yellow colour. He holds it to be a milky excrement	Bayen	—
Meconium (cow)	Contains true gall-like substances	Buniva & Vauquelin	—
Eggs (wild birds)			
Air-space	Does not contain air of different composition from atmospheric air	Hehl	1796
Shells	Phosphate of lime, animal glue, and some combustible substance which escapes with a sulphurous smell from shells when they are softened in acid. Ferrous particles. Sometimes some common salt. An egg, which weighed 2 ozs. 2 scruples 15 grains, had white which weighed 10 *qentchen* 2 scruples, yolk $\frac{1}{2}$ oz. $\frac{1}{2}$ scruple, and shell and membranes 2 drachms 5 grains	von Wasserberg	1780
Shell-membranes	An animal material insoluble in acids	von Wasserberg	1780
Egg-white	6 *qentchen* 2 scruples 7 grains lost practically 6 *qentchen* in drying, it contains no caustic salts, the ash is an earthy insipid dust	von Wasserberg	1780
,,	Albuminous matter, water, muriate of soda, phosphate of lime, and sulphur	Adet	—
Yolk	Albuminous matter, oil, yellow pigment	Adet	—
,,	From 60 eggs, $5\frac{1}{2}$ ozs. oil	Dehne	—
Shell-membranes	Albuminous matter with much oxygen	Adet	—
Shell	Carbonate of lime, phosphate of lime, and very oxydised albuminous matter	Adet	—

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814 (*cont.*)

Substance or liquid investigated	Composition	Investigator	Date
Eggs (domestic hen)			
Shell	A fine earth and a gelatinous material	Macquer	1781
,,	True lime, containing perhaps phosphoric acid	Leonhardi	—
,,	½ oz. of pulverised clean shell, digested with spirits of wine, gave 1½ grains of an extract which smelt and tasted rancid. The same amount of shell gave 1 scruple of a yellow watery extract which tasted salt	Neumann	—
,,	Carbonate and phosphate of lime, traces of a jelly, which can be used as gum. Phosphoric acid can be had from the ash	Berniard	1780
,,	Carbonate and phosphate of lime, bitter earth and iron, a jelly which can be used as gum	Hatchett	1800
,,	Carbonate of lime 72 parts, phosphate of lime 2, jelly 3, water and loss 23	Merat-Gaillot	—
,,	Carbonate of lime 89·6 parts, phosphate of lime 5·7, animal substance 4·7, traces of sulphur. As a hen lays 130 eggs in six months and as an egg weighs on an average 58·117 grams, 7486·226 grams of solid must be used for egg-production in that time, i.e. since the shells would weigh 64·685 gm., 7333·793 gm., 14 pounds 15 ounces 7 gros 8 grains. The secretion of the lime is probably accomplished by means of the kidneys	Vauquelin	1799
,,	Carbonate and phosphate of lime, and jelly	Fourcroy	—
,,	Very much carbonate of lime, very little phosphate. Traces of phosphate of iron, earthy carbonates, muriates, albuminous and gelatinous substance to hold it together. I cannot find any uric acid in it, as Vauquelin says is there, nor is he right in saying that the sulphur is in the shell—it is in the membranes only, and under the form of sulphuric acid	John	1811
Shell-membranes	Consist of an animal material	Macquer	1781
,,	Have the properties of the fibrous part of blood	Jordan	—
,,	A jelly-like material, soluble in hot water	Fourcroy	—
,,	An animal substance with traces of phosphate of lime, carbonate of lime, muriates, and a sulphurous body	John	1811
,,	An albuminous substance containing traces of sulphur and soluble in caustic potash	Vauquelin	—

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814 (*cont.*)

Substance or liquid investigated	Composition	Investigator	Date
Chalazae	An agglutinative substance insoluble in water, apparently like dried tragacanth gum	John	—
Egg-white	A white lymphatic transparent sticky slimy material	Macquer	—
,,	Soda, albuminous matter, water, sulphur	Jordan	—
,,	Water, albuminous matter, with some free alkali, phosphate of lime, muriate of soda, and sulphur	Fourcroy	—
,,	Contains benzoic acid	Proust	—
,,	Water 80 parts, uncoagulable substance 4·5 parts, albuminous matter 15·5 parts, traces of soda, sulphuretted hydrogen gas, and benzoic acid	Bostock	—
,,	Contains sulphur	Scheele	—
,,	Water, albuminous matter, a little jelly, soda, sulphate of soda, muriate of soda, phosphate of lime, oxyde of iron (?)	John	—
Yolk-membrane	An oxydised albuminous substance	Fourcroy	—
,,	Apparently an albuminous substance	John	—
Yolk	Consists of a lymphatic material and a fatty oil	Macquer	1781
,,	Water, oil, albuminous matter, jelly	Thomson	—
,,	Water, oil, albuminous matter, jelly, phosphates of lime and soda, with other salts	Hatchett	—
,,	Water, oil, albuminous matter	Jordan	—
,,	Water, a mild oil, albuminous matter, a colouring matter which is perhaps iron	Fourcroy	—
,,	Water, a yellow mild oil, traces of free (phosphoric?) acid, a small amount of a reddish-brown material, not fatty, and soluble in ether and warm alcohol, a jelly-like substance, a great deal of a modified albuminous substance, and sulphur	John	—
Egg (Snipe, *Tringa vanellus*)			
Shell	Is composed of the same constituents as that of the hen, but the dark green pigment and the dark brown splashes are probably oxyde of iron	John	—
Egg (lizard, *Lacerta viridis*)			
Yolk	A yellow oil, an albuminous material, and salts	John	—
White	Differs from that of fowls in being granular and greasy when hardened by boiling	John	—
Egg (fish, salmon)	420 grains contained of pure dry albuminous matter 26 grains, of a viscous oil 18 grains, insoluble albuminous matter 102 grains, mu-	John	—

EXCERPTS FROM J. F. JOHN'S *CHEMISCHE TABELLEN* OF 1814 (*cont.*)

Substance or liquid investigated	Composition	Investigator	Date
	riate of soda and sulphuric alkali 28 grains, jelly, phosphate of lime, and oxyde of iron 2 grains, water 242 grains		
Egg (fish, *Cyprinus barbus*)	Contains a substance dangerous for man, the nature of which is unknown	Crevelt	—
Egg (insect, *Locusta viridissima*, and *migratoris*)			
Shell	An animal combustible substance and phosphate of lime	John	—
Contents	Albuminous matter, a yellow fluid fatty oil, a little jelly and a characteristic substance, acid, phosphates, and sulphuric alkali	John	—

The most interesting of the investigators in this table is Dzondi, whose work in 1806 was the first in which definite chemical characteristics were systematically followed throughout embryonic development. It is surprising that so long a time should have elapsed between Walter Needham and John Dzondi: no less than 139 years.

After 1814 events were to move so rapidly in the world of science that it would not be possible to follow all the embryological work that was done, and at the same time maintain the proper proportion between the historical part of this book and the other parts. The eighteenth century was the period during which the chemical side of embryology began to differentiate and split itself off from the rest. After 1814 it pursued a course of its own, the individual tracks of which I shall mention under their appropriate heads. But another century had yet to pass before the value of the physico-chemical approach to embryology could become generally recognised, and we are ourselves only at the very beginning of this new period.

A certain contrast may appear between the critical treatment which I have given to the investigators whose work I have been discussing, and the saying of William Harvey's—"all did well", which stands prefixed to this Part of the book. Yet history without criticism is a contradiction in terms, and the praise and dispraise, which I have tried to allot as accurately and justly as I could, is, as it were, technical, rather than spiritual. All the workers who have been mentioned, and others besides them who left no special marks on their time, are worthy of our respect and of our fullest praise, for they preferred wisdom before riches and, according to their several abilities and generations, diligently sought out truth.

PART III

GENERAL CHEMICAL EMBRYOLOGY

All things began in order, so shall they end, and so shall they begin again, according to the ordainer of order and the mystical mathematicks of the city of heaven.

Sir Thomas Browne.

PRELIMINARY NOTE

There have already been certain reviews of work in chemical embryology as a whole, among which those of Paechtner and Schulz are the most valuable. The former dealt almost exclusively with the chemistry of the egg from a static viewpoint, and only devoted a short section to the metabolism of the embryo during its development, while the latter, though dealing specifically with embryonic metabolism, gave hardly more space to it than Paechtner. In both cases the discussion was little more than a catalogue of references, and in neither case was the literature anything like complete, including, indeed, less than a tenth of the relevant citations.

The first review of chemical embryology was written by Gräfe in 1910, but, though he outlined several valuable ideas, it is now of small importance. Good information may, however, be found in Aron's monograph on the chemistry of growth and on the mammalian side there are Harding and Murlin. Other, less satisfactory, reviews are by Cazzaniga and Steudel. Finally there is, of course, an immense amount of work which can be found in no review, for investigators have followed the counsel of Godlevski (1910): "Unsere Kenntnisse hinsichtlich der chemischen Zusammensetztung der Eier noch lange nicht ausreichend sind, so waren weitere Forschungen auf diesem Gebiete auch aus dem Grunde sehr erwunscht weil sie den Ausgangspunkt für die Physiologie des embryonalen Stoffwechsels welcher bisher gleichfalls nur sehr wenig untersucht wurde, bilden mussen".

Every effort has been made to give an accurate and complete presentation of the data in the Tables of this book and of the experimental conditions under which they were obtained, but investigators should always consult in addition, whenever possible, the relevant original memoirs referred to in the Bibliography.

SECTION 1

THE UNFERTILISED EGG AS A PHYSICO-CHEMICAL SYSTEM

1·1. Introduction

In giving an account of the present state of our knowledge about the chemical constitution of the egg-cell and the food-material which is accumulated around it or inside it, I shall not follow a strictly logical order of exposition, according to the phyla of systematic biology. I have judged it best to begin with the egg of the hen, for not only is it the most familiar and the best known of all eggs, but it is also the one which has been most thoroughly investigated biochemically.

It should be remembered that the two main morphological divisions of the egg, (*a*) the egg-cell itself and (*b*) its coverings, appear in protean modifications throughout the animal kingdom. The former may be a simple cell with its ooplasm, nucleus, nucleolus, etc., as in the echinoderms, and no covering at all save its cell-membrane, or at the other extreme it may be swollen up with food-material or yolk to the prodigious proportions of the avian egg-cell. The membrane again may be a thin coat of investing cells such as the tunicate egg possesses, or it may be the jelly of the amphibian egg, or, again, it may be the complex arrangement of egg-white, chalazae, shell-membranes, and shell, which is present in the bird's egg. All imaginable degrees of richness in yolk are present in the egg-cells of animals, and upon this fact depend the various kinds of cleavage which they show: alecithic eggs, on the one hand, such as those of most invertebrates, having a holoblastic form of development in which the whole egg participates in cleavage; and yolk-rich eggs, on the other hand, such as those of most vertebrates, having a meroblastic development, only a localised part of the egg undergoing cleavage, the rest remaining as a sac full of yolk until it is finally absorbed.

1·2. General Characteristics of the Avian Egg

After the historical introduction which has been given, it should be unnecessary to remark on the general arrangement of the bird's egg. We have with Harvey referred to it as an exposed, and, as it were, detached uterus, and with Fabricius ab Aquapendente we have enumerated the parts of the typical avian ovum. Fig. 13, however, shows the general disposition of parts diagrammatically.

First, as to size and shape. The size and shape of the egg were shown by Curtis in 1911 and by Surface in 1912 to be due partly to the structure of the oviduct, which very probably may be considered an inherited character, as was claimed by Newton. D'Arcy Thompson's discussion of the mechanics of egg-formation in birds, in his *Growth and Form*, will be familiar, but some biologists, such as Horwood, have taken exception to his conclusions about the physical influences which shape the egg. Ernst's well-known experiment was the starting-point of these discussions; she caused hens to lay on a surface of wet sand and charcoal, and so, observing the process, found the blunt end to be blackened. This was in agreement with many other observers, such as v. Nathusius; Landois; Jasse; König-Warthausen and Erdmann; and d'Arcy Thompson accordingly described the hen's egg as moving down the oviduct blunt end forwards, the pointed end owing its form to the peristaltic compression of the oviduct. Unfor-

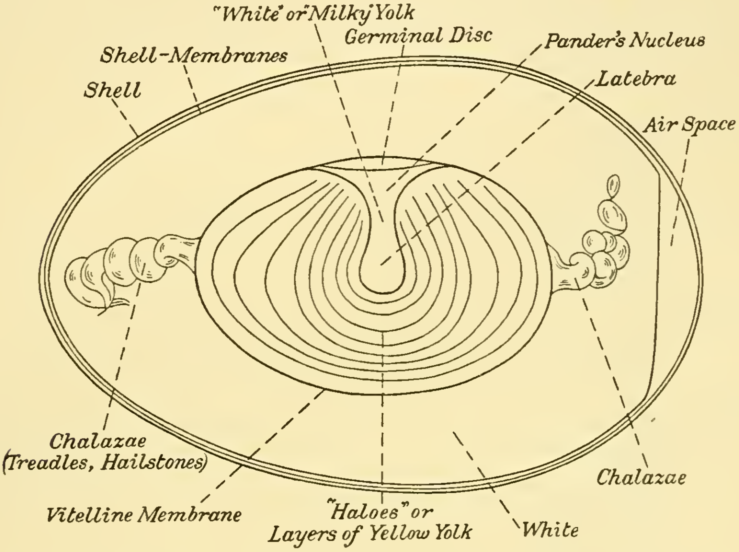

"White" or Milky Yolk
Germinal Disc
Pander's Nucleus
Shell-Membranes
Shell
Latebra
Air Space
Chalazae (Treadles, Hailstones)
Chalazae
Vitelline Membrane
"Haloes" or Layers of Yellow Yolk
White

Fig. 13. Diagrammatic representation of the hen's egg. The chalazae were called by Tredern *Ligamenta albuminis*. Bartelmez gives a discussion of the factors governing the angle which the embryonic axis makes with the axis of the egg as a whole. The yolk is not a perfect sphere but lengthened along the main axis. The egg-white is divisible into three layers which increase in density from without inwards. The chalazae, as Berthold was the first to find, are not present in reptilian eggs.

tunately all observers agree (Purkinje; von Baer; Coste; Kütter; Taschenberg; Wickmann and Patterson for the hen, Blount and Patterson for the pigeon, Kütter for the hawk, and Wickmann for the canary) that the pointed end passes first down the oviduct. It appears that the egg must turn right round in the act of being laid, and Bartelmez, indeed, has seen this occur. Curtis has shown that the shape of the egg depends to some extent upon its size and this biometric observation was afterwards confirmed by Pearl & Curtis.

Many abnormalities have been reported in eggs. They need merely be mentioned here with their authorities, thus:

General.

Hargitt.
Henneguy.
Bartelmez.

Eggs containing masses of tissue, more or less organised.

von Nathusius.
Glücksmann.
Benjamin.

Dwarf eggs.

Pearl & Curtis.
Féré.
Benjamin.

Ovum in ovo.

Herrick.
Chidester.
Weimer.
Rosnatovski.
Camerano.
Pearl & Curtis.
Racah.
Benjamin.
Roberts & Card.
Hilden.

Double and triple-yolked eggs.

Hargitt.
Parker.
Patterson.
Glaser.
Curtis.

Inadequate shell.

Riddle & King.

Dwarf or absent yolk (ovum centennium[1]).

Mercier.
Szuman.
Bugnion.
Gelabert.

---

[1] See Sir Thos. Browne, *Pseudodoxia Epidemica*, Bk III, ch. 7, "Of the basilisk". The eggs of Chelonia also, according to Deraniyagala, are sometimes laid without yolks.

It is interesting in this connection that Riddle has traced the occasional production of eggs with deficiency of white and shell but not of yolk, to a lack of the thymus hormone which he has called "Thymovidine". Feeding with desiccated thymus removed completely these effects. "The whole of the data", he said, "seem to demonstrate the presence in the thymus of a substance having a highly specific action on the oviduct of birds—and presumably on that of all those vertebrate animals which secrete egg-envelopes." The syndrome involved eggs with normal yolks but hardly any shell or albumen, frequent reduction of normally paired ovulations to single ovulations, diminished fertility, and restricted hatchability of the eggs. "Though not necessary to the life of the individual", said Riddle, "thymovidine would seem to be essential to the perpetuation of those vertebrate species whose eggs are protected by egg-envelopes. Such animals were the ancestors of mammals and thus mammals could hardly have come into existence without the thymus." These considerations are of much interest in view of other speculations on the evolutionary aspect of chemical embryology, e.g. Section 6·6. They also suggest that the mammalian thymus is now a vestigial organ.

The air-space, the shell and the white of the normal egg need no special remark at present, but the yolk is a more complicated structure. Around a central core of "white" or "milky" yolk the yellow yolk is secreted in the ovary of the hen in concentric layers, which form the appearance of "haloes" in the finished egg, and which show up especially clearly when the hen is fed on Sudan III or some other non-toxic dye which has a selective staining action on fat. The white yolk in the centre is continued in a flask-like shape (the latebra) up to the surface of the yolk underneath the germinal disc, and is then continued in a very thin layer all round the exterior of the yolk underneath the vitelline membrane. The white yolk is thus the first nourishment of the embryo. It is not certain whether there are also layers of white yolk between the concentric layers of yellow yolk, for they have never been analysed chemically, and Balbiani maintains that they only differ from the yellow layers by having less yellow pigment. The differences between the true white yolk and the yellow yolk are, as will be seen later, far more profound. Balfour & Foster, in their *Elements of Embryology* of 1877, described the yellow yolk as consisting histologically of spheres of from 25 to 100 μ in diameter, filled with numerous minute highly refractive granules and very susceptible to crushing and rough treatment. After boiling, the spheres assume a polyhedral form. The granules seen within them must consist of protein, for they are not soluble in ether or alcohol. On the other hand, the white yolk elements are vesicles smaller than the globules of the yellow yolk, being about 4 to 75 μ across, with a highly refractive body, often as small as 1 μ, in the interior of each. These vesicles are sometimes collected together into much larger vesicles. They observed also underneath the blastoderm or the germinal disc a number of large vacuoles filled with fluid—large enough, in fact, to be seen with the naked eye. The histology of yolk has been reviewed by Dubuisson, and at one time many papers were published on it, e.g. those of Virchow. They cannot be considered in detail here.

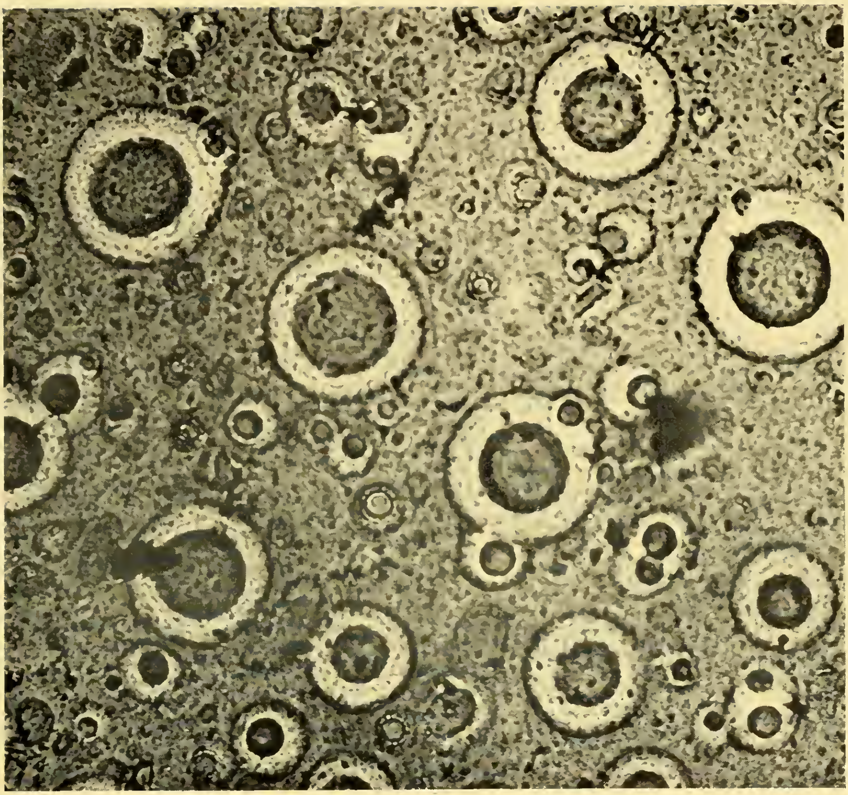
1·3. The Proportion of Parts in the Avian Egg

Of the weight of the whole egg, the shell takes up about 10 per cent., the albuminous white 50 per cent. and the yolk 30 per cent. in round numbers. These relationships have been determined by a multitude of investigators, whose results are drawn up in Table 1.

Table 1. *Distribution of the parts in the egg.*

Italic figures represent dry weight only.

Species	Average egg weights (gm.)	Shell (%)	White (%)	Yolk (%)	Investigator and date
Hen, Polish i ...	43·6	10·34	—	—	van Hamel-Roos (1890)
,, Polish ii ...	56·3	9·75	—	—	,,
,, Holland (Zwol.)	43·8	9·21	—	—	,,
,, Holland (Tiel)	62·6	9·70	—	—	,,
Duck	56·88	9·72	49·50	40·78	Münster Ag. Sta. (1900)
Hen	—	9·39	57·50	33·11	Drechsler (1896)
Duck	—	10·38	58·28	31·34	,,
Hen	—	10·1	59·7	30·2	Plimmer (1921)
Duck	—	11·1	51·0	37·9	,,
Hen	56·70	10·9	57·00	—	Langworthy (1901–2)
Duck	—	13·7	—	—	,,
Goose	157·5	14·2	—	—	,,
Turkey	—	13·8	—	—	,,
Guinea-fowl ...	—	16·9	—	—	,,
Plover	—	9·6	—	—	,,
Hen	50·50	10·89	58·42	30·69	Lebbin (1900)
,, ...	—	11·47	58·33	30·20	Welmanns (1903)
Goose	158·9	11·30	53·40	35·30	Segin (1906)
Duck	67·74	11·40	53·20	35·40	Lührig (1904)
Hen	52·50	12·70	54·40	32·90	,,
Hen (various breeds)	55·58	10·21	55·63	34·15	von Czadek (1917)
Hen	—	10·00	54·38	35·62	Rose (1850)
,, ...	—	—	*33·82*	*66·18*	,,
Hen (various breeds)	—	10·47	56·07	33·46	Carpiaux (1903)
Hen	—	—	54·70	35·30	Lehmann (1850)
,, ...	—	—	*32·30*	*67·70*	Prout (1859)
,, ...	—	—	*37·30*	*62·70*	Poleck (1850)



YOLK OF HEN'S EGG AT THE TIME OF LAYING

Stained by Kossa's method for the detection of calcium phosphate. The considerable variations in the vitelline globules may be noted. Magnification, 6 × D: prepared and microphotographed by Dr V. Marza.

Table I (*cont.*)

Species	Average egg weights (gm.)	Shell (%)	White (%)	Yolk (%)	Investigator and date
Hen, Leghorn ...	58·81	9·21*	—	—	Murray (1925)
Hen (various breeds)	50·65	10·00	60·00	30·00	Iljin (1917)
„	—	—	*30·0*	*60·0*	„
Hen	60·5	12·40	56·20	31·40	van Meurs (1923)
„	56·0	12·50	55·50	32·00	„
„	*15·87*	*27·50*	*22·50*	*50·00*	Voit (1877)
Nidicolous birds					
Gull	—	—	76·20	23·80	Tarchanov† (1884)
Starling	—	—	79·20	20·80	„
Robin	—	—	76·60	23·40	„
Nightingale ...	—	—	75·00	25·00	„
Canary	—	—	73·60	26·40	„
Thrush	—	—	74·40	25·60	„
Raven	—	—	80·00	20·00	„
Corncrake ...	—	—	85·90	14·10	„
Dove	—	—	78·30	21·70	„
Nidifugous birds					
Plover	—	—	61·40	38·60	„
Quail ...	—	—	60·00	40·00	„
Hen	—	—	64·30	35·70	„
Guinea-fowl ...	—	—	54·50	45·50	„
Turkey	—	—	58·30	41·70	„
Duck	—	—	58·30	41·70	„
Goose	—	—	47·40	52·60	„
Pigeon	17·78	6·75	71·10	22·15	Glikin (1908)
	(grains)				
Hen	764·5	10·13	64·51	25·21	Davy (1863)
	—	—	*10·19*	*55·00*	„
Jay	127·3	5·03	68·11	26·86	„
	—	—	*12·80*	*30·40*	„
Hedge-sparrow ...	34·5	5·79	72·46	21·45	„
	—	—	*12·20*	*58·10*	„
Golden-crested wren	14·3	4·90	71·05	24·05	„
	—	—	*9·90*	*43·60*	„
Robin	38·8	5·43	70·33	24·22	„
„	73·8	2·71	75·20	22·08	„
	—	—	*8·20*	*16·50*	„
Missel-thrush ...	124·6	5·13	81·30	12·84	„
Starling	115·1	7·12	78·62	14·25	„
Pigeon	278·0	8·05	73·95	17·94	„
	—	—	*11·50*	*39·30*	„
	(gm.)				
Hen	57·57	11·6	56·8	31·6	Hartung (1902)
„	—	11·9	55·0	33·1	Voit (1881)
„	60·18	—	—	—	Féré (1896)
Duck	73·90	—	—	—	„
Hen	58·38	10·30	89·70		Pott & Preyer (1882)
Rhea	707·0	10·45	89·55		Rozanov (1926)
Goose	133·0	12·72	63·14	36·86	Hepburn & Katz (1927)
Duck	80·3	10·58	61·92	38·08	„
Hen	—	8·89	61·41	29·7	Baudrimont & de St Ange (1846)
Dwarf hen	—	16·88	47·40	35·71	Sacc (1847)
Hen	49·92	—	—	—	Pott (1879)
„	—	11·2	88·8		Atwater & Bryant (1906)

* ±0·11. † All Tarchanov's figures exclude the shell weight.

Table I (*cont.*)

Species	Weight of egg-contents (gm.)	% of whole	Shell (%)	White (%)	Yolk (%)	Investigator and date
Hen	57·12	87·94	12·06	56·22	31·72	Friese (1923)
Goose	137·38	85·95	14·05	53·23	32·72	,,
Duck	78·11	88·05	11·95	50·03	38·02	,,
Turkey	92·93	89·23	10·77	56·72	32·51	,,
Dove	21·88	90·40	9·60	72·35	18·05	,,
Seagull	40·40	91·16	8·84	64·28	26·88	,,
Plover	25·40	91·54	8·46	50·71	40·83	,,
Guinea-fowl ...	41·14	83·40	16·60	44·02	39·38	,,
Pheasant	27·03	90·12	9·88	52·79	37·33	,,
Blackbird	6·98	91·69	8·31	59·46	32·33	,,
Starling	2·98	90·27	9·73	—	—	,,
Canary	1·82	95·05	4·95	58·59	36·26	,,

Species	Weight of egg-contents (gm.)	Shell (%)	White (%)	Yolk (%)	Investigator and date
Plover (*Vanellus cristatus*) ...	22·75	7·5	27·5	68·0	Bauer (1893–5)
Hen (*Gallus domesticus*) ...	48·0	12·0	55·0	33·3	,,
Guinea-fowl (*Meleagris gallopavo*)	73·0	10·9	58·5	29·5	,,
Swallow (*Hirundo rustica*) ...	1·36	11·8	54·0	19·3	,,
Partridge (*Perdrix cinerea*) ...	13·1	12·2	50·0	37·0	,,
Sparrow (*Passer domesticus*) ...	2·7	—	—	—	,,
Thrush (*Turdus* ?) ...	6·4	14·4	56·0	37·0	,,
Duck (*Anas*) [DOUBLE]... ...	101·6	9·6	49·3	35·5	,,

The above data were all obtained without any *ad hoc* investigation of the probable errors involved in weighing eggs and parts of eggs. An elaborate study by M. R. Curtis in 1911 gave the following results on *Gallus domesticus*:

	Actual weight in gm.	%
Whole egg	56·04	100
Albumen	33·22	59·26
Yolk	16·31	29·14
Shell and membranes ...	6·28	11·18
Error	0·23	0·42

But though this is the case with the egg in its natural state, the solid matter is concentrated much more in the yolk than in the white, so that, as the analyses of Poleck and Iljin, for instance, show, for dry weight the conditions are exactly reversed. The egg-white may, indeed, be regarded as the principal reservoir of water for the embryo which develops on dry land, and this is a point which will be discussed later (see Section 6·6). The eggs of different breeds of hen vary to some extent in the relative weights of shell, white and yolk; but, although it is difficult to lay down any general rule, these variations do not greatly exceed the variations due to factors connected with the individual hen. Iljin's lightest shells make up about 7 per cent. of the egg weight and the heaviest not more than 11·5 per cent.

It is certain that there are constant differences between the eggs of different breeds, but as a whole these are quite outweighed by individual differences, and only appear when extended statistical studies are undertaken. The eggs of other birds, however, do not fall within these limits. Langworthy, for example, has shown that, in the duck's egg, the shell may account for as much as 14 per cent. of the whole weight. A similar result was found for the turkey and the goose, while the guinea-fowl's egg has a shell of nearly 17 per cent. of the whole weight. The wide series of Friese, shown in Table 1, seems to indicate that the larger the egg the more shell it has to have: thus the canary's egg weighing just under 2 gm. has 4 per cent. while the goose's egg which weighs 137 gm. has 14 per cent. Heinroth, and Groebbels & Möbert, among others, have collected a great many data of this kind for all varieties of bird, but their papers must be referred to for the figures. Thus the fertilised embryo starts its development on the surface of a mass of food only slightly diluted with water, and surrounded by a further and much wetter supply. This is reflected well by the work of Bellini, who found that the yolk of the hen's egg was seven times as viscous as the white at the beginning of development. (Alb. 3·4 units, yolk 28·5 units.)

A good deal of work has been done on the variability of the weights of the parts of the egg within a given species of fowl. Thus Jull found that egg weight is the least variable factor, albumen weight slightly more variable than egg weight, yolk weight considerably more variable than albumen weight, and shell weight the most variable. It would seem, therefore, as if a compensatory process takes place during egg-production, the largest yolks having the smallest whites, since the weights of the entire eggs do not vary as much as the weights of the components. On the other hand, the smaller eggs contain the highest percentage of albumen and shell and the lowest percentage of yolk. Jull also studied closely the seasonal variations, which may be quite considerable, finding that the component parts of the egg contribute in different degrees at different times of the year towards the total egg weight. The question as to which part of the egg is mainly responsible for large or small eggs is still debated, for Curtis concluded from his observations that it is the egg-white, while Atwood found many indications contrary to this. Statistical studies on the egg of the tern have been made by Rowan, Parker & Bell; Rowan, Wolff, Sulman, Pearson, Isaacs,

Table 2. *Composition of avian eggs (whole).*

Italic figures represent data in which the shell is included.

Species	Per cent. wet weight: Water	Per cent. wet weight: Protein	Per cent. wet weight: Fat	Per cent. wet weight: Non-nitrogenous extractives	Per cent. wet weight: Ash	Per cent. dry weight: Protein	Per cent. dry weight: Fat	Per cent. dry weight: Nitrogen	Weight of egg	Weight of egg-contents	Investigator and date
Hen	72·75	16·53	10·72	—	—	—	—	—	—	—	Prévost & Morin (1846)
,, ...	72·46	11·36	13·40	1·73	1·05	41·25	48·66	6·60	60·4	53·2	König & Farwick (1876)
,, ...	73·99	13·71	11·27	—	1·03	52·71	43·33	8·43	—	—	Commaille (1873)
,, ...	74·64	13·63	10·43	—	1·34	53·75	41·13	8·59	—	—	Payen (1863)
,, ...	73·61	11·49	13·36	0·46	1·08	43·54	50·63	7·01	49·2	39·3	König & Krauch (1878)
,, ...	72·50	—	—	—	—	—	—	—	—	—	Amar (1924)
Duck	71·11	12·24	15·49	—	1·16	42·37	53·62	6·78	59·8	52·1	Commaille (1873)
Hen	—	—	—	—	0·91	—	—	—	—	—	Buckner & Martin (1919)
Plover	74·43	10·75	11·66	2·19	0·98	42·04	45·78	6·73	24·9	22·5	König & Krauch (1878)
Hen	73·7	13·4	10·5	—	1·0	—	—	—	—	—	Langworthy (1901)
,, ...	*65·5*	*11·9*	*9·3*	—	*0·9*	—	—	—	—	—	,,
,, ...	*65·6*	*11·8*	*10·8*	—	*0·6*	—	—	—	—	—	,,
,, ...	*64·8*	*11·9*	*11·2*	—	*0·7*	—	—	—	—	—	,,
Duck	70·5	13·3	14·5	—	1·0	—	—	—	—	—	,,
,, ...	*60·8*	*12·1*	*12·5*	—	*0·8*	—	—	—	—	—	,,
Goose	69·5	13·8	14·4	—	1·0	—	—	—	—	—	,,
,, ...	*59·7*	*12·9*	*12·3*	—	*0·9*	—	—	—	—	—	,,
Turkey	73·7	13·4	11·2	—	0·9	—	—	—	—	—	,,
,, ...	*63·5*	*12·2*	*9·7*	—	*0·8*	—	—	—	—	—	,,
Guinea-fowl ...	72·8	13·5	12·0	—	0·9	—	—	—	—	—	,,
,, ...	*60·5*	*11·9*	*9·9*	—	*0·8*	—	—	—	—	—	,,
Plover	74·4	10·7	11·7	—	1·0	—	—	—	—	—	,,
,, ...	*67·3*	*9·7*	*10·6*	—	*0·9*	—	—	—	—	—	,,
Hen	*65·16*	*11·67*	*10·30*	—	*0·93*	*33·5*	*29·58*	—	50·50	45·00	Lebbin (1900)
,, ...	72·40	12·6	11·37	—	1·02	—	—	—	—	—	Mansfeld (1901)
Hen, Dutch "kippen" breed	74·79	10·81	10·50	—	0·87	42·88	41·65	—	—	—	Greshoff, Sack, van Eck & Bosz (1902)

Duck	68·00	12·00	9·24	—	4·04	37·50	27·00	—	68·50	61·0	,, ,,
Plover	75·30	11·00	10·26	—	0·89	44·53	41·54	—	—	—	,, ,,
Hen	—	—	—	—	—	50·68	42·57	—	—	—	Welmanns (1903)
Goose	—	—	—	—	—	—	—	—	158·9	140·9	Segin (1903)
Duck	—	—	12·20	—	—	—	—	—	67·74	60·02	Lührig (1906)
Hen	—	—	11·05	—	—	—	—	—	52·5	45·80	,,
,, ...	73·98	—	—	—	—	—	—	—	—	—	Chapin & Powicke (1904)
,, ...	74·34	—	—	—	—	—	—	—	—	—	,, (1915)
Hen, average of many different breeds ...	—	13·82	11·76	—	—	—	—	—	55·58	45·99	von Czadek (1917)
Hen, Leghorn ...	75·30	11·70	11·10	—	—	47·50	45·00	7·6	—	—	Murray (1925)
Yellowhammer ...	81·40	—	—	—	—	—	—	—	—	—	Groebbels* (1927)
Nightingale... ...	80·00	—	—	—	—	—	—	—	—	—	,,
Blackbird	80·11	—	—	—	—	—	—	—	—	—	,,
Carrion crow ...	83·31	—	—	—	—	—	—	—	—	—	,,
Pewit	61·28	—	—	—	—	—	—	—	—	—	,,
Hen	73·7	14·8	10·5	—	1·0	—	—	—	—	—	Atwater & Bryant (1906)
,, ...	—	12·50	11·37	—	—	—	—	—	—	—	Sakuragi (1917)
,, ...	73·31	—	—	—	—	—	—	—	—	—	Hartung (1902)
,, ...	76·94	11·30	10·86	—	0·85	50·50	47·10	8·08	49·70	—	Liebermann (1888)
,, ...	—	—	—	—	0·55	—	—	—	—	—	Pott & Preyer (1882)
Starling	77·40	—	—	—	—	—	—	—	2·79	—	Tangl (1903)
Hen, Plym. Rock ...	75·50	—	—	—	—	—	—	—	58·16	52·00	,,
,, Hungarian ...	74·20	—	—	—	—	—	—	—	52·20	46·20	,,
,, Dorking ...	74·00	—	—	—	—	—	—	—	55·30	49·70	,,
,, Plym. Rock ...	75·12	—	11·36	—	—	—	—	—	48·46	44·08	Tangl & v. Mituch (1908)
,, ,, ...	—	—	—	—	—	—	—	—	59·60	53·64	,,
Starling	—	—	—	—	—	—	28·26	—	—	—	Glikin (1908)
Hen	—	—	11·50	—	—	—	—	—	—	—	Idzumi (1924)
,, ...	73·80	—	—	—	1·15	—	—	—	—	—	Pott & Preyer (1882)
Goose	71·25	14·12	12·20	—	1·17	—	—	—	—	—	Hepburn & Katz (1927)
Duck	68·55	13·64	14·36	—	0·82	—	—	—	—	—	,,
Hen	*68·4*	*16·3*	*9·1*	—	*5·8*	—	—	—	—	—	Baudrimont & de St Ange (1846)

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* For a wide range of data on the weights of eggs and shells only, see Heinroth; Groebbels & Möbert and Langworthy.

Elderton & Tildesley; and by Watson, Watson, Pearson, Karn, Irwin & Pearson.

The division of birds into the two classes of nidicolous (those which hatch as squabs) and nidifugous (those which hatch as downy, feathered and active chicks) has been shown to extend to the composition of their eggs by several investigators. Davy found that the eggs of the nidicolous birds had thinner and more fragile shells, which took up a less proportion of the weight of the whole egg than the shells of nidifugous birds. Thus the wren's egg-shell weighs only 5 per cent. of the whole egg weight, while the hen's weighs 10 per cent. Davy's figures show very clearly that the main reservoir of solid is the yolk and the main reservoir of water is the egg-white.

Tarchanov carried the matter further, and observed that the yolks of nidicolous birds always formed a smaller proportion of the total amount of material inside the egg than in nidifugous birds. Thus, for the former class the egg-white accounts for about 78 per cent. of the egg and the yolk for 22 per cent., while, in the latter class, the egg-white accounts for about 55 per cent. and the yolk for 45. All these differences are probably related to the shorter incubation-time of the nidicolous eggs; and, as will be seen later, there are not wanting indications that the yolk of these is less tightly packed with food-material and more rich in phosphatides.

1·4. The Chemical Constitution of the Avian Egg as a whole

The composition of the egg as a whole is further considered in Table 2, where it is noticeable that the analyses of water and ash have not been significantly improved upon between 1863 (the date of the first analysis, Payen's) and the present time. The later figures for protein and fat are, however, much the more reliable. It should be observed that there is an approximately equal quantity of fat and protein at the disposal of the embryo, though the former is, of course, in the yolk, and the latter is preponderantly in the egg-white. This protein-fat equality is by no means the rule in all eggs, and, as we shall see later, the eggs of fishes depart widely from it. There appear to be only small differences between the eggs of different kinds of birds in protein content. At one time it was thought that the duck's egg was particularly rich in fat, on the authority of Commaille's analyses, but Lührig has since then brought it into line with all the others. It does seem, however, to have a considerably higher percentage of mineral substances than the rest. The dry-weight figures merely demonstrate again the approximate equality of the protein and fat.

Before we proceed to consider the parts of the egg in separation, the question of individual and racial differences must be taken up

Table 3. *Individual differences between hen's eggs.*

Malcolm's figures (1902). Averages of individual hens.

	Fatty acids	Nitrogen	P_2O_5
Breed unknown	27·79	3·00	1·49
	29·45	2·62	—
Italian hens (fed on maize and barley)	28·50	2·65	1·36
	28·66	2·67	1·53
	31·89	2·68	1·40
	28·96	3·12	1·52
	29·11	2·87	1·51
	30·08	2·67	1·57
	30·12	2·82	1·50
	29·56	2·84	1·65
From one hen	31·04	2·57	1·39
	31·57	2·55	1·42
	30·43	2·61	1·44
From one hen	30·12	2·75	1·48
	30·26	2·75	1·51

Iljin's figures (1917).

	In % dry weight		
	Fat	Lecithin P	P_2O_5
Houdan...	56·79	0·33	1·13
Orpington	56·68	0·57	1·10
Plymouth Rock	57·17	0·71	1·13
Rhode Island	59·16	0·62	1·10

Table 4. *Race differences in hen's eggs.*

Leveque & Ponscarme's figures.

	Mean weight of whole egg	gm. dry weight			% of egg dry weight	
		Shell	White	Yolk	P_2O_5 in yolk	N in white
Andalusia	64·7	9·14	7·05	16·32	2·91	18·26
Berrichonne ...	66·3	8·06	8·18	14·10	3·04	18·42
Bressane	60·8	8·41	8·00	13·15	2·88	18·55
Coucou de Rennes	60·3	10·66	7·41	17·07	3·02	18·50
Crèvecœur ...	62·5	8·86	8·95	12·73	2·93	18·14
Dorking	52·4	9·09	9·47	15·57	2·99	18·44
Faverolles	56·3	8·84	8·31	14·80	2·90	18·46
Houdan	57·8	8·95	8·06	13·76	2·99	18·48
La Flêche	62·2	9·20	8·23	14·70	3·02	18·53
Minorca	63·6	8·99	8·17	14·98	2·96	18·60
Orpington ...	57·3	9·03	7·75	15·23	2·86	18·36

Table 5. *Race differences in hen's eggs.*

von Czadek's figures (1917).

Breed of hen	Egg weight	Shell weight	Egg-white weight	Yolk weight	% of whole egg			gm. per egg		% egg-white, wet weight					% yolk, wet weight					Spans of weight
					Shell	Egg-white	Yolk	Protein	Fat	Water	Protein	Fat	Carbohydrate	Ash	Water	Protein	Fat	Carbohydrate	Ash	
Sulmtal	56·71	6·20	32·96	17·55	10·9	58·1	30·9	7·51	5·56	85·92	12·43	0·07	0·70	0·88	46·93	19·47	31·62	0·59	1·39	1·9
	58·61	5·72	35·08	17·81	9·8	59·9	30·4	7·30	5·90	85·92	11·91	0·05	1·24	0·88	46·68	17·50	33·02	1·40	1·40	
Minorca	75·14	6·55	47·87	20·72	8·7	63·7	27·6	9·23	6·79	85·89	12·26	0·09	1·32	0·82	48·57	16·20	32·54	1·20	1·54	1·1
	74·01	6·30	47·07	20·64	8·5	63·6	27·8	9·51	7·02	84·89	12·35	0·09	1·84	0·83	44·92	18·00	33·90	1·64	1·49	
Orpington	43·43	5·21	20·31	17·96	12·0	46·7	41·3	5·95	5·50	82·66	14·98	0·31	1·13	0·92	49·69	16·20	30·33	2·03	1·75	15·1
	58·52	5·54	34·42	18·56	9·5	58·7	31·7	7·44	6·22	86·02	12·35	0·07	0·70	0·86	47·40	17·20	32·55	1·33	1·52	
Rhode Island	45·49	4·87	25·92	14·70	10·7	57·0	32·3	4·93	4·48	87·64	9·68	0·21	1·58	0·89	50·92	16·44	30·12	0·89	1·63	6·4
	51·90	4·50	28·97	18·43	8·7	55·8	35·5	5·93	5·53	88·02	10·01	0·29	0·92	0·96	50·32	16·43	29·58	1·87	1·80	
Faverolle	53·04	5·54	29·54	17·96	10·4	55·7	33·9	6·36	5·52	86·86	11·80	0·14	0·30	0·89	50·86	16·03	30·45	1·30	1·36	9·7
	62·72	6·51	35·50	20·71	10·4	56·6	33·0	7·33	6·35	86·80	10·66	0·09	1·42	1·03	50·82	15·81	30·56	1·17	1·64	
Italian hen	56·88	5·53	30·77	20·58	9·7	54·1	36·2	6·56	6·22	86·17	11·42	0·18	1·28	0·95	51·90	14·82	29·92	1·83	1·53	5·1
	51·73	4·80	28·10	18·83	9·3	54·3	36·4	6·25	5·74	86·50	11·32	0·11	1·22	0·84	49·73	16·28	30·32	2·07	1·60	
Rhineland hen	42·80	5·23	20·45	17·12	12·2	47·8	40·0	5·62	5·29	83·45	14·06	0·27	1·30	0·92	49·67	16·06	30·57	2·04	1·66	6·4
	49·16	4·75	25·11	19·24	9·7	51·2	39·1	7·08	6·19	82·76	14·72	0·54	0·96	1·02	48·40	17·54	31·50	0·88	1·68	
Wyandotte	51·59	6·54	26·36	18·69	12·7	51·1	36·2	6·67	5·83	83·16	13·79	0·42	1·93	0·70	50·94	16·23	30·63	0·68	1·52	
Lowest	42·80	4·50	20·31	14·70	8·5	46·7	27·6	4·93	4·48	82·66	9·68	0·05	0·30	0·70	44·92	14·82	29·58	0·59	1·36	
Highest	75·14	6·55	47·87	20·72	12·7	63·7	41·3	9·51	7·02	88·02	14·98	0·54	1·93	1·03	51·90	19·47	33·90	2·07	1·80	
Average	55·58	5·59	31·23	18·63	10·2	55·6	34·1	6·91	5·88	85·49	12·25	0·20	0·87	0·87	48·19	16·68	31·17	1·39	1·57	

Tangl's figures (1908).

	Egg weight	Egg-shell weight (dry)	In % whole egg weight					In % shell weight				Actual amounts (gm.)			
			Dry weight	Organic substance	Ash	Calcium	Magnesium	Organic substance	Ash	Calcium	Magnesium	Organic substance	Ash	Calcium	Magnesium
Plym. Rock March 20–31	60·86	5·70	9·36	4·36	4·98	3·45	0·081	46·70	53·30	37·00	0·86	2·65	3·03	2·10	0·049
Apr. 8–May 23	58·60	5·83	9·95	4·60	5·36	3·70	0·083	46·20	53·80	37·40	0·84	2·69	3·14	2·17	0·049
Average	59·73	5·76	9·65	4·48	5·17	3·57	0·081	46·40	53·60	37·20	0·85	2·67	3·08	2·13	0·049

Iljin's figures (1917).

	Breed of hen	Egg weight	Shell weight	Egg-white weight	Yolk weight	% egg-white water	% yolk water
Eggs of nearly the same weight	Houdan	51·23	4·40	31·44	15·39	86·97	48·10
	Orpington	51·10	3·43	31·99	16·68	85·61	47·35
	Plym. Rock	51·10	5·57	30·04	15·49	85·66	52·45
	Rhode Island	51·12	4·38	29·55	17·19	87·07	48·31
	Houdan	53·26	5·88	32·23	15·14	87·18	49·65
	Orpington	53·26	3·92	33·98	15·47	85·21	48·61
	Plym. Rock	53·29	5·75	32·57	14·97	86·33	49·31
	Rhode Island	53·32	3·81	32·84	16·66	86·16	50·72
Average for each breed	Houdan	56·28	5·50	34·40	16·39	87·63	49·52
	Orpington	55·50	4·67	33·09	17·73	86·07	49·30
	Plym. Rock	56·64	5·75	34·65	16·27	86·32	49·59
	Rhode Island	58·65	4·02	35·57	16·06	87·53	49·52
	Lowest	55·50	4·02	33·09	16·06	86·07	49·30
	Highest	58·65	5·75	34·65	17·73	87·63	49·59
	Average	57·07	4·88	33·87	16·89	86·85	49·44

Pennington's figures (1910).

		% egg-white wet weight				% yolk wet weight			
		Water	N	Fat	Ash	Water	N	Fat	Ash
Plym. Rock	Highest ...	88·90	1·82	0·050	0·80	49·37	2·76	33·33	2·58
	Lowest ...	87·20	1·62	0·006	0·35	45·88	2·59	31·44	1·31
	Average ...	87·98	1·70	0·024	0·63	47·84	2·68	32·70	1·77
White Leghorn	Highest ...	88·09	1·71	0·04	0·73	47·86	2·83	33·19	2·18
	Lowest ...	87·39	1·64	0·01	0·47	47·07	2·76	31·63	1·46
	Average ...	87·86	1·68	0·02	0·64	47·44	2·79	32·65	1·90

Willard, Shaw, Hartzell & Hole's figures (1909).

Breed of hen	Egg weight	Wt. reqd. to perforate shell in uniform conditions (gm.)	% of whole egg			% white		% yolk					% whole egg			Ash/H_3PO_4 ratio in yolk
			Shell	Yolk	White	Water	Protein	Water	Protein	Fat	Ash	Phosphoric acid	Water	Protein	Fat	
American Red ...	53·75	170·0	9·57	33·38	56·81	88·14	11·86	48·39	17·18	32·87	1·58	1·43	65·79	12·55	10·98	1/1·09
Plym. Rock ...	52·25	179·0	10·09	32·99	56·72	88·10	11·91	48·74	17·75	31·97	1·55	1·38	66·20	12·62	10·51	1/1·11
White Leghorn ...	51·77	182·0	10·46	32·59	56·55	87·42	12·58	48·76	17·83	31·80	1·55	1·43	65·55	12·97	10·53	1/1·09
White Wyandotte ...	55·50	185·0	9·77	32·16	57·95	87·06	12·95	48·58	17·50	32·37	1·54	1·42	66·05	13·11	10·41	1/1·08

again. Malcolm maintained in 1902 that, although there were undoubtedly differences between the eggs of different breeds of hen, they did not exceed the amount of variation between individual eggs from hens of the same breed. His results are shown in Table 3. Thus, although the feeding was carefully controlled, the eggs from one hen might show a difference of 1·14 gm. of fat, while between two eggs from different breeds the difference might be only 0·13 gm. His conclusions were supported in the main by Carpiaux; Leveque & Ponscarme; von Czadek; Iljin; and Willard, Shaw, Hartzell & Hole; who made very long studies of a considerable number of breeds. Some of the figures obtained by von Czadek and Iljin are given in Table 5. Von Czadek studied the Sulmtal, Minorca, Orpington, Rhode Island, Faverolle, and Wyandotte races, together with an Italian and a Rhineland breed. His outside values for egg weight, for instance, were 43 gm. and 75 gm.—a considerable difference—but the former was from the Rhineland hen and the latter from the Minorca variety. The span showed great variations, thus an egg weighing 55 gm. might be a heavy Orpington or a rather light Faverolle or a medium weight Italian. The only breed which stood well out of the range of individual differences was the Minorcas which laid very heavy eggs. Certain instances have shown, however, that remarkable agreement may exist between work done on eggs of widely different breeds. Thus the classical work of Plimmer & Scott on the phosphorus metabolism of the developing chick was confirmed very strikingly by Masai & Fukutomi, who worked in Japan. Here the correspondence was almost numerical. But on the other hand there is evidence that eggs of different breeds differ not only in their gross characteristics, but also as regards more subtle properties; thus Moran has demonstrated that eggs from different breeds of hen vary very greatly in their resistance to cold, so that the viability is different, and Needham, working on the inositol metabolism of the embryo, observed differences between the embryos from Black and White Leghorn hens. Physico-chemical differences between breeds of silkworm eggs are enumerated by Pigorini.

The individual differences between eggs may be equally important. Benjamin has shown that there are numerous variable factors which modify the constitution of the egg. The amount of yolk, egg-white, and water, as well as the thickness of the shell, vary according to the season, diet, age (Riddle) and general condition of the bird in question. Nor are such comparatively slowly changing factors the only ones which bring about differences between individual eggs; the time the egg takes to pass down the oviduct, for instance, will materially affect the amount of albumen it contains, and such variable quantities as the blood-sugar level (Riddle) and the level of cholesterinaemia in the parent animal will exercise their effects upon the resulting egg. Again, the length of time elapsing between the laying of the egg and the beginning of incubation will have a marked effect, for a certain amount of water will evaporate from the egg-contents through the shell, and just how much does so will depend on the humidity of the surrounding atmosphere. The process of water-absorption by the yolk (Greenlee) from the white will also be affected by these conditions, so that the embryo at the initiation of its incubatory development may find a remarkably inconstant set of circumstances in its immediate environment. Moreover, a certain amount of development always takes place in the egg after fertilisation as it passes down the oviduct, so that the embryo has already gastrulated by the time that the egg is laid by the hen. It was the ignorance of these facts which led Malpighi, as we have already seen, to his erroneous conclusions, for if he had known of the phenomenon of "body-heating", as it is called by the poultry-farmer, he would not have put forward the preformation-theory, and the eighteenth century would have been spared the trouble of getting rid of that embryological phlogiston. Thus no two eggs are ever exactly the same age, and as there is reason to believe that enzymic action begins in the yolk, if not in the white, very shortly after fertilisation, this fact makes it additionally difficult to get precise figures for the constitution of the unincubated egg. Then the position of the egg in the clutch (whether first or second) in pigeons may, according to Riddle, make a difference of 9–15 per cent. in yolk weight. It may be concluded that nothing short of the greatest caution must be employed in the material which is used for chemico-embryological researches on the hen's egg. The individual hens should be marked, and the eggs produced by them should be noted, their food should be constant in composition and the breed used should be not only single in any one series of experiments, but also, if possible, genetically pure. It is very greatly to be wished that standard hens could be obtained, such as the standard rats necessary for feeding experiments, and much further work, with a proper statistical backing, is needed on the range of individual and racial variations in all the properties of eggs.

The effect of the diet of the hen on the chemical composition of the egg has been studied by various workers, notably by Terroine & Belin. Except in certain respects, it showed a remarkable fixity of composition:

Table 6.

	Ordinary mixed ration	Corn and potato ration almost free from fats	Hemp seed ration (fatty)
White in % of total weight	56·7	54·3	—
Yolk in % of total weight	31·3	34·0	33·2
Shell in % of total weight	11·4	10·9	—
White			
Water %	87·8	87·4	87·4
Ash %	0·49	—	—
Yolk			
Water %	49·9	50·33	50·99
Ash %	1·48	—	—
Total nitrogen %	2·67	—	—
Total fatty acids %	28·4	26·6	26·55
Unsaponifiable fraction %	1·85	—	2·08
Cholesterol %	1·18	1·58	1·11
Lecithin P %	—	0·425	0·434

Thus, although the character of the substances stored for the use of the embryo can be varied considerably, as will be seen later, the balance of them cannot. But the question is probably rather complicated, for it has been shown by Dam that by feeding hens on a ration rich in cholesterol, the cholesterol content of eggs can be increased from 501 to 615 mgm. per cent. of the wet weight or roughly by 22 per cent. of the original value. In another instance the cholesterol rose from 476 to 560 mgm. per cent. This would not be in disagreement with Terroine & Belin's figures, but it would be a very desirable thing to make a detailed study of the limits of variation of all the constituents of the egg, and to find out exactly how different in chemical composition an egg can be from the normal while retaining its hatchability. Klein regards the cholesterol output of the hen in its eggs as showing a synthesis of that substance in the parent body. Leveque & Ponscarme have stated that it was not possible to show any effect on the eggs in eleven breeds of hen by minor variations in the diet; and this was amply confirmed by Cross.

The ingenious and partially successful attempt of Riddle and Behre & Riddle to make hens preserve their own eggs by feeding them with hexamethylenetetramine, sodium benzoate, and sodium salicylate, may here be mentioned. Starting out from this practical suggestion the work led to the discovery of a number of specific effects of substances such as quinine on egg size and yolk size. Thus Riddle & Basset found that alcohol markedly reduces yolk size in pigeons, Riddle & Anderson found that quinine reduces egg size, yolk size and albumen size but has no effect on the protein/fat ratio of the egg, while Behre & Riddle found that the diminution of albumen size under quinine bore more on the solids than on the water and involved considerable reduction of the protein.

The elaborate investigations on the egg of the tern, already mentioned, led to a significant correlation between abundance of food and size of egg, and it is certain that the size of the hen's egg is affected by its diet since the work of Atwood. There seems also to be a seasonal fluctuation, the weight of the eggs increasing from July to February and decreasing from March to June. These seasonal fluctuations appeared distinctly in Atwood's data, and explain the results of Curtis and of Féré. Rice, Nixon & Rogers and Riddle found a definite relation between the amount of food consumed and the number of eggs produced, both of these factors varying exactly with the seasonal variation in the egg size. Fluctuations of a regular kind seem even to occur each month, according to Hadley who observed such changes in egg weight and number. According to Curtis the size of the eggs increases as the laying bird matures, in the case of the hen, and Pearson has observed similar variations in the case of the sparrow.

The genetics of egg production have been studied by Pearl and Benjamin.

The relation between the egg weight and the chick weight at hatching has been studied by Halbersleben & Mussehl and by Iljin. The former workers found a quite consistent relation within one breed between the weight of the egg before incubation and the weight of the chick at hatching, the latter averaged 64 per cent. of the former. After thirty-five days of post-natal life, however, the slight advantage possessed by the chicks from the heavier eggs had altogether disappeared. They also noted that, other things being equal, chicks hatched from the more pigmented eggs (browner) weighed slightly more than those hatched from the less pigmented ones. Abnormally large and abnormally small eggs did not hatch as well as those of medium weight. Iljin collected a great many figures but his text contains no statistical analysis.

Stewart & Atwood reported that chicks hatched from pullet eggs were neither so large nor so vigorous as those hatched from the eggs of hens two or three years old. Whether there is here a direct effect on the chick of the age of the hen, or whether the effect is indirect, due to the small size of the egg, may be well questioned.

What relations exist between the chemical constitution of the egg and the percentage "hatchability" are at present obscure, owing perhaps to the comparative crudity of our estimation methods. The work of Pearl & Surface indicated definitely that differences in the hatchability of eggs are determined by or associated with innate differences in the individual hens which laid them, that these differences are probably inherited, and that variations within rather wide limits in certain environmental factors, e.g. the temperature, during incubation, are of secondary importance in determining the death or the hatching of the embryo. Hatchability of embryos would appear then to be, like fecundity, a heritable character. The experiments of Lamson & Card confirmed the conclusions of Pearl & Surface, but although some physico-chemical mechanism is undoubtedly at work, these statistical studies gave no hint as to its nature.

Dunn determined to probe further into it. In his first paper he argued that if hatchability was associated with constitutional vigour, it should show a correlation with such a value as the chick mortality in the first three weeks of post-natal life. Experimentally this was not the case, e.g. post-natal mortality remained the same, although in two instances the pre-natal mortality was on the one hand extremely high (20–39 per cent. hatchability) and on the other hand extremely low (80–100 per cent. hatchability). It therefore seemed likely that mortality before and after hatching is determined by quite different factors. The more specific influences operating in embryonic life must doubtless be looked for in the physico-chemical constitution of the unincubated egg.

Hays & Sumbardo, in a search for such influences, were able to exclude statistically fresh weight, length, diameter, specific gravity, shell thickness, outer and inner shell-membrane thickness, porosity and imbibition of water from 25 per cent. salt solution. Other factors which have been excluded are percentage of protein in the diet of the laying hen (Rosedale), percentage of yolk-pigment (Benjamin), evaporation rate of the egg (Dunn), yolk-fat percentage (Cross), egg-fat percentage (Cross), yolk-protein percentage (Cross), egg-protein percentage (Cross), egg-phosphorus percentage (Cross), chick-phosphorus percentage (Cross).

It appears, however, that the constitution of the egg-proteins may be influenced by the presence of unusual proteins in the diet of the hen, and that this may influence hatchability. Pollard & Carr have reported the results of feeding the following proteins to laying hens: wheat, rye, corn, oats; kaffir, barley, peas, soya, hemp; buckwheat, popcorn, sunflower seed.

The first group of four (all, of course, being fed alone) were very efficient for the production of normal eggs; the second group (of five) permitted the hens to lay eggs but the eggs were hardly hatchable at all, while the third group allowed of no eggs. Pollard & Carr studied the egg-proteins in all cases and obtained evidence of tryptophane deficiency in the second group, so that they concluded that a minimum tryptophane content was essential for successful development through hatching. It is unfortunate that their results were never published in full.

The effect of sex on the chemical composition of the egg has been discussed by Riddle. As is well known, in some, probably most, animals, the male produces two kinds of spermatozoa which are not equal in their prospective sex value, i.e. some which will give rise to females and some which will give rise to males. In birds, on the other hand, the dimorphism of the germs exists not in the spermatozoa but in the egg-cells. The female produces two kinds of eggs of unequal prospective sex value. Riddle found that pure wild species of doves and pigeons were ideal material for studies on sex, since very abnormal sex-ratios could easily be obtained from them, and his studies led him to the view that sex was more a matter of metabolic level or rate of protoplasmic activity than anything else. But what concerns us here are the consistent differences which he was able to demonstrate between male and female eggs.

Pigeons generically crossed, when not permitted to lay many eggs, produce only males, but when made to lay many eggs produce first only males, and eventually "under stress of overwork" only females. These facts and their proper conditions having been ascertained previously by extensive statistical investigations, the way lay open for the chemical analysis of the two sorts of pigeon's eggs. 900 analyses were made and more than 12,000 yolks weighed.

Fig. 14, taken from Riddle, gives the differences diagrammatically. A glance at it shows that the male-producing egg of the spring contains less stored material than the female-producing egg of the autumn. The amount of water and ash present, on the other hand, diminishes, and the rise indeed is mainly to be seen in the fat and lipoid fractions and in the calorific value. Table 7 gives the figures for one individual pigeon during 1912. The differences are not

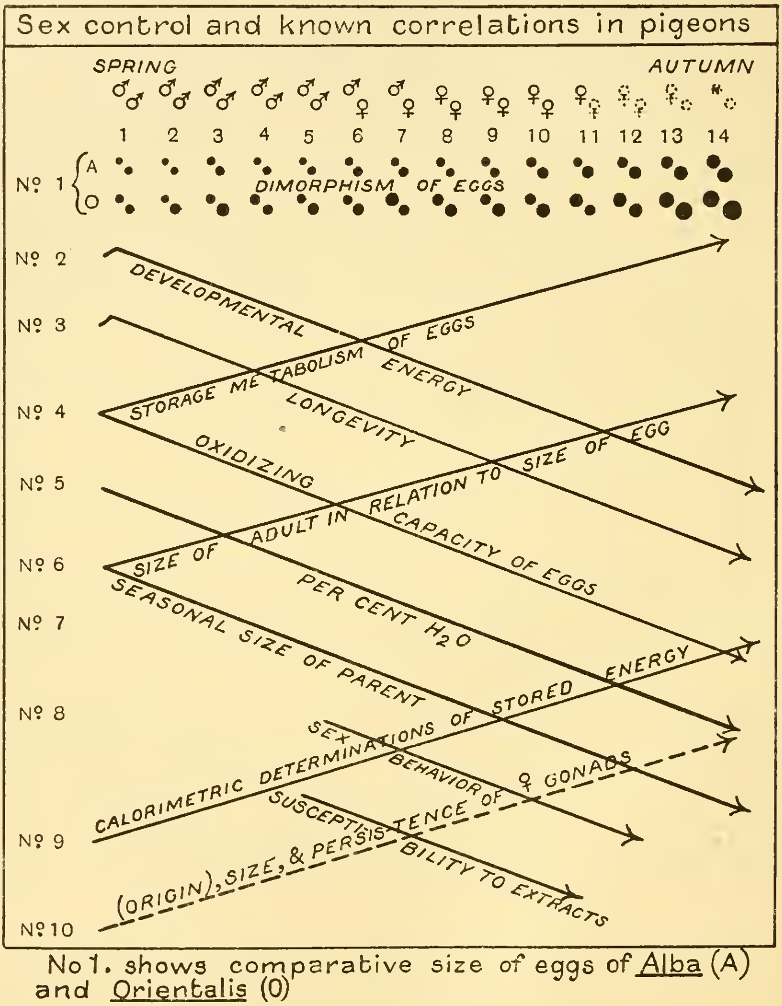

Sex control and known correlations in pigeons
SPRING
AUTUMN
1 2 3 4 5 6 7 8 9 10 11 12 13 14
No. 1
A
O
DIMORPHISM OF EGGS
No. 2
No. 3
No. 4
No. 5
No. 6
No. 7
No. 8
No. 9
No. 10
DEVELOPMENTAL ENERGY
STORAGE METABOLISM OF EGGS
LONGEVITY
OXIDIZING CAPACITY OF EGGS
SIZE OF ADULT IN RELATION TO SIZE OF EGG
PER CENT H2O
SEASONAL SIZE OF PARENT
CALORIMETRIC DETERMINATIONS OF STORED ENERGY
SEX BEHAVIOR
SUSCEPTIBILITY TO EXTRACTS
(ORIGIN), SIZE, & PERSISTENCE OF ♀ GONADS
No 1. shows comparative size of eggs of Alba (A) and Orientalis (O)

Fig. 14.

large, but they were invariably found. Another series of figures showing the rise in calorific value during the course of the year and the transition from male to female eggs is given in Table 8. Here also the increase is unmistakable. Within one clutch, also, the water-content of the second egg is lower and the calorific value higher than the first egg, which fits in very well with the fact that under normal

Table 7. *Effect of sex on pigeon's eggs.*

Female *Turtur orientalis* × *Streptopelia alba*, no. 410 for 1912.

			% wet weight					% dry weight		
Date	Analysed or incubated	Weight of yolk	Lipoid	Protein	Extractives	Ash	Water	alc.-sol.	Calories per egg	Sex
May 26	An.	2·330	18·32	25·44	5·28	4·85	57·01	72·65	7405	—
28	An.	2·660	17·54	25·63	5·25	2·62	54·82	72·45	8990	—
June 7	Inc.	—	—	—	—	—	—	—	—	Male
15	Inc.	—	—	—	—	—	—	—	—	Male
26	Inc.	—	—	—	—	—	—	—	—	Male
July 3	An.	2·026	16·49	26·00	3·63	2·43	56·05	71·95	6714	—
5	An.	2·330	19·18	26·55	3·75	1·93	55·22	72·27	7881	—
15	Inc.	—	—	—	—	—	—	—	—	Male
17	Inc.	—	—	—	—	—	—	—	—	Male
23	An.	2·422	17·82	25·88	3·82	1·80	55·84	72·42	8061	—
25	An.	2·720	18·88	25·96	3·86	1·81	55·33	72·45	9296	—
Aug. 2	Inc.	—	—	—	—	—	—	—	—	Male
4	Inc.	—	—	—	—	—	—	—	—	Male
13	Inc.	—	—	—	—	—	—	—	—	Male
25	Inc.	—	—	—	—	—	—	—	—	Female
Sept. 15	Inc.	—	—	—	—	—	—	—	—	Female
17	Inc.	—	—	—	—	—	—	—	—	Female
Nov. 29	An.	2·700	21·40	—	—	—	55·45	73·17	9323	—
Dec. 1	An.	2·715	21·63	—	—	—	55·39	73·02	9383	—

Table 8. *Eggs from the same female* Streptopelia risoria (1914).

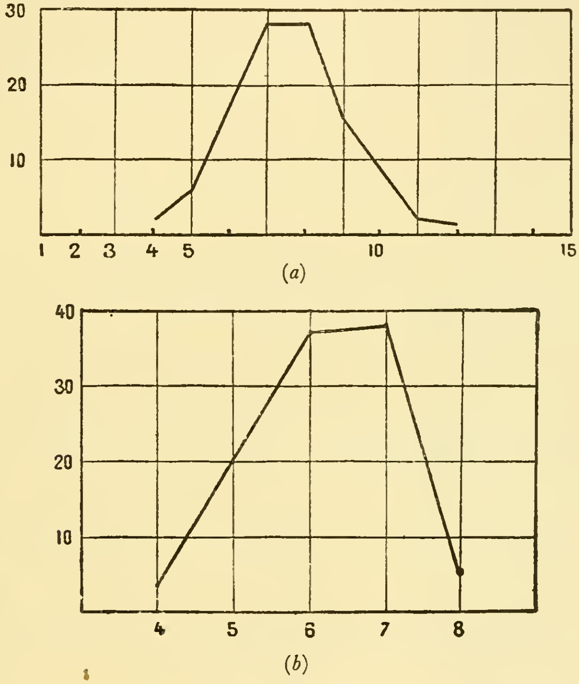
Date	Weight of yolk	Energy in cals.
June 6	1·010	3358
8	0·970	3175
19	0·855	2807
21	1·000	3245
July 14	1·145	3815
16	1·463	5008
Aug. 30	1·395	4812
Nov. 6	1·440	4837
8	1·720	5797
20	1·590	4906
22	1·780	6015
Dec. 1	1·640	5614
3	1·820	6255
12	1·535	5302
14	1·690	5601
23	1·485	5266
25	1·718	5880

conditions the first egg laid nearly always gives rise to a male and the second to a female.

In Fig. 14 the line marked "developmental energy" implies that a higher percentage of the male eggs hatch successfully than of the female eggs. The data for length of life show the same curve. The smaller eggs of both clutch and season are the eggs which give positive results in strength and vigour tests, and the larger eggs are those which are liable to display weakness. These facts are in entire accord with the higher metabolic level which Riddle associates with the small male eggs. It is interesting to note that Lawrence & Riddle found consistently higher values for total fat and total phosphorus in the blood of female fowls than in that of male fowls, from which they concluded that the metabolic differences between male and female germs persist in the adult, and all these facts are in agreement with the work of Goerttler and Baker on human and Smith on crustacean blood-fat, and of Benedict & Emmes on sex differences in basal metabolism. But for further discussion of the metabolic theory of sex, the papers of Riddle must be consulted. Interesting data on the hatchability, vigour, etc., of rotifer eggs are contained in the paper of Jennings & Lynch, but these authors made no chemical experiments.

To say, as Riddle does, that there are, as it were, two kinds of eggs in some species, one male-producing, and the other female-producing, may either be taken to mean that there are quantitative differences between them or that their constituent substances are qualitatively chemically different, or, thirdly, that the same substances in the same quantities are differently distributed spatially and temporally. As will be seen later in connection with the lipoids of mammalian egg-cells, the second view finds supporters, and some such opinion is held by Russo. Fauré-Fremiet, in the course of his work on the egg of *Ascaris megalocephala*, to which he applied every conceivable method, examined a very large number of individual eggs in order to find whether they separated at all chemically into two types. His method was to centrifuge them separately, much as McClendon had done with the frog's egg, and then to measure in mm. the thickness of (*a*) the mitochondria layer, and (*b*) the fatty layer. Fig. 15 (*a*) taken from his paper shows the frequency polygon which he constructed on the basis of these results, the ordinate giving the number of eggs measured, and the abscissa the thickness of the mitochondrial layer.

It is quite evident that there are not two modes on the line joining the points, i.e. that there are not two types of eggs, but only one type. Fauré-Fremiet made very similar experiments, determining the glycogen content of the eggs histo-colorimetrically with iodine solutions, and there also the frequency polygon had but one mode (Fig. 15 (*b*)). But this second case was based on an unsatisfactory method. In the particular instance under investigation, neither mitochondria nor glycogen happens to be an entity which varies as between the two kinds of eggs. Nevertheless, the plan of work was an interesting one, and widely extended researches with it, using accurate chemical methods, would be very desirable.



(*a*)

(*b*)

Fig. 15.

1·5. The Shell of the Avian Egg

Little attention has been paid to the shell of the bird's egg from a physiological point of view. The relevant analyses are given in Table 9. There is some difference between the shells of different

Table 9. *Composition of shell* (%).

BIRDS	$CaCO_3$	$MgCO_3$	$Ca_3(PO_4)_2$	P_2O_5	K	Organic substance	Water	SiO_2	Al_2O_3	Ca	Investigator and date
Hen	93·7	1·3	0·8	—	—	4·2	—	—	—	—	Langworthy (1901)
Goose	95·3	0·7	0·5	—	—	3·5	—	—	—	—	,,
Duck	94·4	0·5	0·8	—	—	4·3	—	—	—	—	,,
Hen	97·91	0·76	—	0·66	—	—	—	—	—	—	Buckner & Martin (1920)
Kingfisher (*Ardea cinerea*)	94·6	0·69	—	0·42	—	4·3	—	—	—	—	Wicke (1863)
Herring gull (*Larus argentatus*)	91·96	0·76	—	0·83	—	6·45	—	—	—	—	,,
Pheasant (*Phasianus colchicus*)	93·33	0·66	—	1·37	—	4·64	—	—	—	—	,,
Goose (*Anser domesticus*)	95·26	0·72	—	0·47	—	3·55	—	—	—	—	,,
Hen (*Gallus domesticus*)	93·7	1·39	—	0·76	—	4·15	—	—	—	—	,,
Duck (*Anas domesticus*)	94·42	0·5	—	0·84	—	4·24	—	—	—	—	,,
Hen (various breeds)	92·95	—	—	0·44	—	5·53	—	—	—	—	Carpiaux (1903)
Hen	—	—	—	0·43	0·29	—	—	—	—	—	Browning (1917)
Ostrich	97·44	—	—	—	—	3·28	—	—	—	—	Wicke (1856)
Ostrich (second century A.D.)	94·14	0·69	1·82	—	—	3·05	0·15	—	—	—	Balland (1881)
Ostrich (modern)	91·44	2·03	0·70	—	—	4·92	0·73	—	—	—	,,
Ostrich	95·74	2·14	0·44	—	—	1·10	0·34	0·06	0·13	—	Torrance (1923)
Hen	93·90	—	—	—	—	6·13	—	—	—	52·5	Voit (1877)
Hen	—	—	—	—	—	4·20	—	—	—	—	Kelly (1901)
Ostrich	—	—	—	—	—	3·37	—	—	—	—	,,
REPTILES											
Alligator (*Alligator sclerops*)	91·10	2·33	0·54	—	—	5·09	1·36	—	—	—	Wicke & Brummerstad (1855)
Snake (*Coluber natrix*)	14·40	—	—	—	—	—	—	—	—	—	Neumeister (1895)
Crocodile (*Crocodilus biporcatus*)	78·00	2·60	—	—	—	—	—	—	—	—	,,
Gecko (*Ptychozoon homalops*)	—	—	—	—	—	1·07	—	—	—	—	,,
Tortoise	—	—	7·30	—	—	—	—	—	—	—	Gmelin (1901)
Alligator (*Alligator sclerops*)	—	—	—	—	—	4·2	—	—	—	—	Kelly (1901)
MOLLUSC											
Land-snail (*Bulimen oblongata*)	—	—	—	—	—	4·5	—	—	—	—	,,

birds, and it would be interesting, for example, to know why the pheasant's contains such an unusually high percentage of phosphorus, and why the herring gull's has so high a percentage of organic substance. A certain interest attaches to the determinations of Balland on ostrich eggs, some from a tomb of the Hellenistic period and others from modern ostriches, but the differences he found were probably not very significant, as the analysis of Torrance seems to give values half-way between those of Balland. Neither Balland, Torrance nor Wicke states whether the ostrich used was the North or South African variety, a complication which might make a difference. Wicke believed that the difference in shell-composition between different kinds of birds was almost entirely dependent on their usual foods.

The microscopic structure of the shell was investigated by Nathusius in the 'sixties, and since then little has been added to his work. The shell consists of an outer layer of crystals of calcium carbonate arranged with their long axes perpendicular to the bounding surface (Fig. 16), and an inner layer composed of undifferentiated calcium carbonate (Herzog & Gonell). Kelly; Schmidt; Meigen and Osawa have found that the mineralogical form of the lime is invariably calcite, no aragonite being present in any bird's egg-shell. This has been confirmed with X-ray analysis by Mayneord. The ostrich, *Emys europaea*, is the only doubtful case, for Kelly identified its egg-shell lime as conchite, but Torrance considers it to be calcite. Prenant's review should be consulted for further details regarding this interesting biochemical problem. Only one paper exists dealing with the changes which the shell undergoes histologically during the development of the chick; it will be considered in the section on embryonic respiration, where the data we possess on the question of the

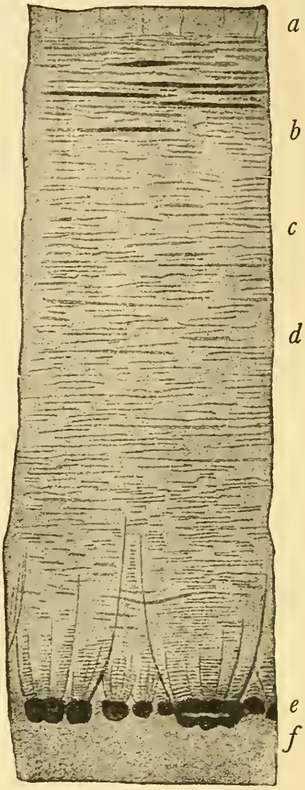


Fig. 16. *a*, Outer crystalline layer; *b*, *c*, *d*, amorphous layers; *e*, mamillae; *f*, shell-membrane.

Table 10 *a*. *Elementary constitution of Sauropsid egg-proteins.*

Species and protein	C	H	N	S	P	O	$(a)^D$ optical rotation	Investigator and date
BIRDS: Hen								
Total ovoglobulin*	51·91	7·07	15·13	2·00	—	23·89	—	Langstein (1903)
Soluble globulin*	51·43	7·04	15·16	1·66	—	24·71	—	,,
Ovoeuglobulin	50·04	7·11	14·40	1·75	—	26·70	—	,,
Ovoalbumen (cryst.)	52·46	7·19	15·29	1·34	—	23·72	—	,,
,, (not cryst.)	52·23	6·96	15·98	1·75	—	23·08	—	,,
Ovomucoid	48·82	6·90	12·41	2·19	Trace	29·78	—	,,
Ovoalbumen (cryst.)	—	—	—	1·64	—	—	—	Osborne (1899)
,, ,,	—	—	—	1·11	—	—	—	Hofmeister (1890)
Ovolivetin	—	—	15·20	1·80	0·067	—	—	Kay (1928)
Ovovitellin	51·24	7·16	16·38	1·04	0·94	23·24	—	Osborne & Campbell (1900)
Ovomucidin	50·69	6·71	14·49	2·28	—	25·83	—	,, ,,
Ovoalbumen	52·75	7·10	15·51	1·62	0·12	22·30	−29·4 to −30·7°	,, ,,
Conalbumen	52·25	6·99	16·11	1·70	—	22·95	−36·0 to −39·0°	,, ,,
Ovomucoid	49·02	6·45	12·71	2·38	—	29·44	−61·35°	,, ,,
Hydrolysis products of ovoalbumen								
Protalbinic acid	55·4	7·2	14·3	2·4	—	—	—	Gupta (1909)
Lysalbinic acid	52·9	7·0	14·0	1·2	—	—	—	,,
Lysalbinpeptone	46·2	6·6	10·3	1·2	—	—	—	,,
Ovoalbumen (cryst.)	—	—	—	—	0·13	—	—	Wilcock & Hardy (1909)
Ovovitellin	—	—	—	—	0·99	—	—	Plimmer (1908)
Ovolivetin	—	—	—	—	0·35	—	—	,,
Ovomucoid	—	—	12·65	2·20	—	—	—	Mörner (1894)
,,	—	—	12·47	2·27	—	—	−70·9°	Mörner (1912)
Ovochromin	42·6	6·7	8·08	1·60	—	70·78	—	Barbieri (1912)
Ovovitellin	48·01	6·35	14·91–16·97	0·88	0·33	—	—	Gross (1899)
Ovoalbumen	52·90	7·11	15·8	—	—	—	—	Wurtz (1902)
Serum albumen	53·1	7·1	15·9	1·9	—	—	—	Bywaters (1909)
Ovoalbumen	52·5	7·2	15·3	1·3	—	—	—	,,
Ovomucoid	48·8	6·9	12·4	2·2	—	—	—	,,
Seromucoid	47·5	6·8	11·75	1·8	—	—	—	,,
Mucin	48·8	6·8	12·3	0·8	—	—	—	,,
Ovovitellin	51·60	7·22	15·02	—	—	26·16	—	Dumas & Cahours (1842)
,,	—	—	—	1·0	—	—	—	Schwarzenbach (1867)
Shell-membrane keratin	49·78	6·64	16·43	4·25	—	—	—	Lindwall (1881)
Split-products of vitellin								
Paranuclein	44·60	6·66	14·51	0·88	3·35	—	—	Levene & Alsberg (1900)
Avi-vitellic acid	32·31	5·58	13·13	0·32	9·88	—	—	,,
Ovomucoid	47·06	7·53	10·29	3·50	1·65	—	—	Milesi (1898)
,,	48·85	6·92	12·46	2·22	—	—	—	Zanetti (1897)
Ovoalbumen	—	—	—	—	0·92	—	—	Kaas (1906)
Ovofibrinogen	52·85	7·02	15·71	—	—	—	—	Gautier (1902)
Keratin of vitelline membrane	46·21	7·55	12·20	3·62	—	30·42	—	Liebermann (1888)
Chalaza-protein	48·26	8·07	—	—	—	—	—	,,
,,	47·94	9·81	—	—	—	—	—	,,
Keratin of shell-membrane	50·95	7·24	Present	Present	—	—	—	,,
,, ,,	50·60	6·60	16·70	—	—	—	—	Scherer (1856)
Ovovitellin	52·26	7·25	15·06	1·17	None (!)	25·43	—	Gobley (1846)
,,	—	—	—	0·42	1·02	—	—	Baumhauer (1866)
,,	—	—	—	0·75	None (!)	—	—	Avonheim (1866)
,,	52·02	—	12·60	—	—	—	—	Zadik (1898)
Turkey: Ovoalbumen	52·97	7·39	15·37	1·60	—	22·67	−34·9°	Worms† (1906)
Dove: Ovoalbumen (columbin)	52·17	7·16	14·82	—	—	—	−36·33°	Panormov (1905)
Duck: Ovoalbumen i, (anatin)	50·32	6·84	14·64	2·96	—	—	−81·95°	,,
Ovoalbumen ii, (anatidin)	52·15	7·31	14·94	2·01	—	—	−37·09°	,,
Ovomucoid from different kinds of birds								
Garrot (*Clangula glaucion*)	—	—	13·12	3·36	—	—	−88·2°	Mörner (1912)
Eider-duck (*Somateria mollissima*)	—	—	13·21	3·33	—	—	−72·7°	,,
Pelican (*Phalaracrocorax carbo*)	—	—	12·52	—	—	—	−74·3°	,,
Guinea-fowl (*Meleagris gallopavo*)	—	—	12·41	2·36	—	—	−74·0°	,,
Grebe (*Podiceps cristatus*)	—	—	11·40	2·37	—	—	−67·1°	,,
Hen (*Gallus domesticus*)	—	—	12·47	2·27	—	—	−70·9°	,,
REPTILE								
Marine turtle (*Thalassochelys corticata*) ovomucoid	48·26	7·08	12·90	2·90	—	—	—	Takahashi (1929)

* A mixture consisting mainly of ovomucein.
† Worms (1903) found three ovoalbumens in crow's eggs (*Corvus frugilegus*), corvin, corvinin, and corvinidin.

Table 10 *b*. *Elementary constitution of egg-lipoids.*

Hen	C	H	N	P	N/P ratio	O	Investigator and date
Ovolecithin	—	—	2·505	3·61	1/1·50	—	Wintgen & Keller (1906)
,,	—	—	1·85	—	1/1·12	—	Trier (1913)
,,	64·18	10·60	1·88	3·95	1·05/1	—	McLean (1909)
,,	64·63	10·96	1·79	3·95	1/1·00	—	Stern & Thierfelder (1907)
Ovokephalin	65·66	11·54	1·37	3·96	1/0·77	—	,, ,,
,,	60·00	9·62	1·78	3·69	1/1·07	—	Levene & West (1916)
Ovocuorin	59·12	9·44	0·812	4·00	1/1·50	27·05	McLean (1909)
Ovosphingomyelin	65·56	11·68	3·84	4·22	2·01/1	—	Levene (1916)
,,	68·15	12·14	2·77	3·22	1·90/1	—	Stern & Thierfelder (1907)
A monoaminodiphosphatide from yolk	—	—	0·815	3·62	1/2·00	—	McLean (1908)

permeability of the shell and its membranes will also be dealt with. There has been some controversy on the subject of whether the egg-shell contains any elements of the secreting organ in it, like the decidua of mammals. Von Baer thought not, but the presence of cellular structures has been reported by von Hemsbach; Landois; and Blasius.

The shell-membrane has been studied chemically by Liebermann and Lindwall, who found that it consisted almost entirely of a protein, the percentage composition of which agreed very closely with keratin (Table 10*a*). Krukenberg, alone, on the ground of its reactions, held it to be a mucin. This ovokeratin, which contains four times as much sulphur as the albumen of the egg-white, was found by Mörner to include 7 per cent. of cystine, but there are reasons for supposing that this figure is much too low. Nothing is known of the part played by ovokeratin in embryonic metabolism, but, in view of the fact that calcium is transported from the shell to the embryo during the period of ossification of embryonic cartilage, it is not impossible that the sulphur or the cystine of ovokeratin may be made use of in a similar manner to meet the need for sulphur and cystine for the feathers. This will be discussed later under the head of sulphur metabolism (Section 12·7). Mörner considered that sulphur must exist in the ovokeratin in other forms besides cystine, for that amino-acid would not account for more than a third of what he found was there. The amino-acid analyses of ovokeratin are placed in Table 11; they are due to Abderhalden & Ebstein, and to Plimmer & Rosedale, the former by isolation and the latter by the van Slyke nitrogen distribution method. The arginine figure is rather high.

The strength of the shell is clearly an important biological factor: according to Romanov its average thickness is 0·311 mm. giving a breaking-strength of 4·46 kilos. The relation between shell-thickness and breaking-strength is a straight line.

The physiological properties of the shell of the bird's egg have been very insufficiently studied. In the last century there was a general impression that the shell possessed a differential permeability and that, while water and other liquids would readily go through the egg-shell and its membranes from outside in, they would not easily pass from the inside to the outside. It is difficult to find how this idea originated; thus Ranke in 1872 attributed it to the younger Meckel, and Ranke's own statement was subsequently copied down

Table 11. *Distribution of amino-acids in egg-proteins.*

	Osborne, Leavenworth & Brautlecht; Osborne, Jones & Leavenworth and Osborne & Gilbert: OVOALBUMEN (crystallised)*	Abderhalden & Pregl and Abderhalden: OVOALBUMEN (crystallised)*	Hugounenq & Morel and Mörner (cystine): OVOALBUMEN (coagulated)†	Skraup & Hummelberger and Herzfeld (tryptophane)				Abderhalden & Hunter and Hedin (arginine): OVOVITELLIN	Osborne, Leavenworth & Brautlecht and Osborne & Jones: OVOVITELLIN	Abderhalden: CASEIN (cow)	Hugounenq: OVOVITELLIN	Zeller: OVOMUCOID	Folin & Denis: OVOVITELLIN
				OVOALBUMEN	PROTALBINIC ACID	LYSALBINIC ACID	LYSALBINPEPTONE						
Glycine	None	None	None	—	—	—	—	1·1	None	None	Trace	—	—
Alanine	2·22	8·10	8·40	—	—	—	—	Trace	0·75	0·9	Trace	—	—
Valine	2·50	—	—	—	—	—	—	2·4	1·87	1·0	1·5	—	—
Leucine	10·71	7·10	15·20	—	—	—	—	11·0	9·87	10·5	6·8	—	—
Proline	3·56	2·25	1·10	1·50	2·0	1·0	0·3	3·3	4·18	3·1	0·5	—	—
Phenylalanine	5·07	4·40	5·20	5·80	12·0	5·2	2·4	2·8	2·54	3·2	0·7	—	—
Aspartic acid	2·20	1·50	1·70	—	—	—	—	0·5	2·13	1·2	0·7	—	—
Glutamic acid	9·10	8·00	3·50	3·20	1·8	1·0	1·6	12·2	12·95	10·7	0·9	—	—
Serine	—	—	—	—	—	—	—	—	—	0·2	Trace	—	—
Tyrosine	1·77	1·10	0·99	2·40	3·4	2·6	1·1	1·6	3·37	4·5	2·0	—	5·2
Cystine	—	0·20	0·40	—	—	—	—	—	—	0·06	—	—	—
Histidine	1·71	—	—	1·50	2·3	0·3	0·6	—	1·90	2·59	2·2	3·88	—
Arginine	4·91	—	—	2·90	0·4	0·2	0·3	2·3	7·46	4·84	1·0	4·29	—
Lysine	3·76	—	0·27	3·90	3·3	5·3	4·0	—	4·81	5·8	1·2	2·42	—
Ammonia	1·34	—	—	—	—	—	—	—	1·25	—	1·17	1·17	—
Glucosamine	1·23	—	—	—	—	—	—	—	—	—	—	—	—
Tryptophane	Trace	—	—	0·52	—	—	—	—	Trace	1·5	—	—	—
Unidentified amino-acids	—	—	—	7·90	14·7	7·0	3·2	—	—	—	—	—	—
Total di-amino N ...	3·20	—	—	—	—	—	—	—	4·36	—	—	—	—
Total mono-amino N ...	—	—	—	—	—	—	—	—	—	—	—	—	—
Non-amino N in di-amino fraction	—	—	—	—	—	—	—	—	—	—	—	—	—

* Acid hydrolysis. † Baryta hydrolysis.

Table 11. *Distribution of amino-acids in egg-proteins* (cont.).

			Plimmer & Rosedale								Folin & Denis		
	Plimmer & Lowndes: MIXED EGG-PROTEINS (van Slyke)*	Abderhalden & Ebstein and Mörner (cystine): OVOKERATIN (shell-membrane) (isolation)	YOLK-PROTEINS (van Slyke)	EGG-WHITE PROTEINS (van Slyke)	SHELL-MEMBRANE PROTEINS (OVOKERATIN) (van Slyke)	OVOMUCOID (van Slyke)	Levene & Alsberg: OVOVITELLIN (isolation)	Chapman & Petrie: OVOALBUMEN	Hugounenq & Galimard: OVOALBUMEN (coagulated)	Abderhalden: OVOMUCOID	OVOALBUMEN	CONALBUMEN	Harris: OVO-ALBUMEN
Glycine	—	3·9	—	—	—	—	Trace	—	—	—	—	—	—
Alanine	—	3·5	—	—	—	—	0·16	—	—	—	—	—	—
Valine	—	—	—	—	—	—	—	—	—	—	—	—	—
Leucine	—	7·4	—	—	—	—	3·30	—	—	4·0	—	—	—
Proline	—	4·0	—	—	—	—	4·00	—	—	2·4	—	—	—
Phenylalanine	—	—	—	—	—	—	1·00	—	—	4·0	—	—	—
Aspartic acid	—	1·1	—	—	—	—	0·60	—	—	1·8	—	—	—
Glutamic acid	—	8·1	—	—	—	—	1·00	—	—	2·0	—	—	—
Serine	—	—	—	—	—	—	—	—	—	—	—	—	—
Tyrosine	—	None	—	—	—	—	0·40	—	—	Traces	5·0	4·9	—
Cystine	1·8 + 3·5	7·6	—	—	—	—	—	—	—	—	—	—	1·51
Histidine	0·40	—	3·1	0·2	4·3	—	Trace	0·66	None	—	—	—	—
Arginine	14·3 + 4·4	—	14·5	11·7	16·9	10·9	1·20	2·39	2·14	—	—	—	—
Lysine	11·0	—	9·4	10·1	9·4	11·7	2·40	3·19	2·15	—	—	—	—
Ammonia	—	—	—	—	—	—	—	—	—	—	—	—	—
Glucosamine	—	—	—	—	—	—	—	—	—	—	—	—	—
Tryptophane	—	—	—	—	—	—	—	—	—	—	—	—	—
Unidentified amino-acids	—	—	—	—	—	—	—	—	—	—	—	—	—
Total di-amino N ...	25·8	—	27·1	22·1	30·7	22·7	—	—	—	—	—	—	—
Total mono-amino N ...	64·0	—	62·1	67·6	62·2	64·3	—	—	—	—	—	—	—
Non-amino N in di-amino fraction	11·0	—	13·1	8·9	15·5	7·4	—	—	—	—	—	—	—

* The figures before the plus signs in the analyses of Plimmer & Lowndes indicate the contents of the diamino fraction; those after them refer to the monoamino fraction.

Table 11. *Distribution of amino-acids in egg-proteins* (cont.).

	Osborne, Leavenworth & Brautlecht: CONALBUMEN	Sendju: MIXED EGG-PROTEINS*	Adensamer & Hoernes: OVO-ALBUMEN	Abderhalden & Kempe: MIXED EGG-PROTEINS	Komori: OVOMUCOID	Kay & Marshall		Levene & Beatty: OVOALBUMEN	Matsuyama & Mori (tryptophane) and Sullivan (cystine): OVOALBUMEN	Jones, Gersdorff & Moeller		
						OVOVITELLIN	OVOLIVETIN			OVOALBUMEN	CONALBUMEN	OVOVITELLIN
Glycine	—	—	None	1·20	—	—	—	2·0	—	—	—	—
Alanine	—	—	Much	—	—	—	—	—	—	—	—	—
Valine	—	—	Present	—	—	—	—	—	—	—	—	—
Leucine	—	—	Present	—	—	—	—	17·0	—	—	—	—
Proline	—	—	—	—	—	—	—	0·5	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	—	—	—
Aspartic acid	—	—	—	—	—	—	—	—	—	—	—	—
Glutamic acid	—	—	—	12·80	—	—	—	8·75	—	—	—	—
Serine	—	—	—	—	—	—	—	—	—	—	—	—
Tyrosine	—	6·72	—	1·82	—	5·0	5·2	1·25	—	—	—	—
Cystine	—	2·56	—	—	—	1·4	3·9	—	0·98	0·88	3·37	0·83
Histidine	2·17	2·14	—	—	4·0	—	—	—	—	—	—	—
Arginine	5·07	4·80	—	—	0·27	—	—	—	—	—	—	—
Lysine	6·43	7·36	—	—	0·97	—	—	—	—	—	—	—
Ammonia	1·17	—	—	—	—	—	—	—	—	—	—	—
Glucosamine	—	—	—	—	—	—	—	—	—	—	—	—
Tryptophane	—	2·14	—	—	—	1·6	2·14	—	1·18	2·25	5·03	2·42
Unidentified amino-acids	—	—	—	—	—	—	—	—	—	—	—	—
Total di-amino N ...	—	—	—	—	—	—	—	—	—	—	—	—
Total mono-amino N ...	—	8·64	—	—	—	—	—	—	—	—	—	—
Non-amino N in di-amino fraction	—	—	—	—	—	—	—	—	—	—	—	—

* These figures apply to acids, not nitrogen.

wrongly by Schäfer. However, Thunberg in 1902 conclusively demonstrated the error of the belief, and showed experimentally that water would pass through the membranes equally well both ways, though he found that of the two the inner one was the less permeable. In the case of water birds, there is evidence that the shell is absolutely impermeable to water; Loisel, for instance, found that the eggs of the grebe, *Podiceps cristatus*, and the duck, *Anas domesticus*, when placed in distilled water absorbed not a trace of it, and gave out no chloride to it over a period of many hours. The eggs of the ordinary hen, on the other hand, increased considerably in weight and allowed some chloride to pass out into the water. At the same time, Loisel found that hen's eggs develop quite normally, at any rate up to the seventh day, even if they are lying in water. This experiment of Loisel's was confirmed by Lippincott & de Puy, who succeeded in hatching chicks from eggs incubated while lying in ½ in. of distilled water. The differences between these eggs and the controls suggested that the eggs lying in the water not only failed to lose as much water through evaporation as eggs incubated under ordinary conditions, because of the limitation of the evaporating surface, but actually absorbed some water. Trials with rhodamine red and methylene blue demonstrated penetration by these dyes, extending in the former case to vital staining of the embryo. It is known (Rizzo) that the avian egg-shell has many pores (0·86 to 1·44, average 1·23 per sq. mm.).

As regards gases, the only paper is that of Hüfner. Hüfner placed small pieces of egg-shell with their membranes in a diffusiometer, and measured the rate at which gases passed through the obstacle. He found that oxygen diffused through with most difficulty, then nitrogen, then carbon dioxide, and, finally, hydrogen most easily. It may be significant that carbon dioxide would thus appear to be able to escape somewhat quicker than oxygen can enter. But under normal atmospheric pressure the amount diffusing through the whole egg-shell (goose) per second was 2·115 c.c. of oxygen and 0·503 c.c. of carbon dioxide. The diffusion velocity was always proportional to the partial pressure of the gases, and the removal of the inner membrane made no difference at all, suggesting that the principal barrier was the amorphous calcium carbonate layer. It would be very desirable to repeat these observations with more modern methods, and on a greater variety of eggs.

1·6. The Avian Egg-white

The white of the egg is divisible into three portions which have been studied separately by Romanov. The outermost and thinnest layer makes up 39·8 % of the whole and has 11·6 % dry solid. The middle layer accounts for 57·2 % and has 12·4 % dry solid, and the innermost, thickest, layer is only 3 % and has 14·5 % dry solid*. The chalazae have only once been analysed separately (Liebermann), when the elementary composition of their protein was ascertained. Table 12 summarises the results of the investigators who have made general analyses of the white. It is a very watery solution of protein, containing only the most negligible traces of fats and lipoids, but a great many water-soluble substances such as carbohydrate in various forms, protein breakdown-products, choline, inositol, etc. Natural egg-white, according to Rakusin & Flieher, is a saturated solution of ovoalbumen (15·35 per cent.). The water-content does not vary much, but Tarchanov's analyses go to show that the smaller eggs with short incubation-time are wetter than the others. The proteins of the egg-white are believed to be variable in number in the eggs of different birds. In that of the hen, four are known, in that of the crow three, and in that of the dove one only. The egg-white of the hen's egg contains two albumens, ovoalbumen and conalbumen, and two glucoproteins, ovomucoid and ovomucin.

It was at one time thought that there was a fifth, ovoglobulin. Dillner studied it in 1885, and estimated that it made up 0·67 per cent. of the egg-white and 6·4 per cent. of the total protein, but Osborne & Campbell showed that it was simply a mixture of the others in different proportions. This had already been made probable by the results obtained by Corin & Berard, who were able to separate the ovoglobulin into two or three constituent proteins having several different coagulation temperatures (57·5°, 67°, 72°, 76° and 82° C.) and other special characteristics.

Hofmeister was the first to prepare crystalline ovoalbumen, and he published several papers on it. Other workers confirmed his discovery, such as Gabriel; Harnack; and Bondzynski & Zoja, but Hopkins & Pincus showed that the albumen so crystallised only accounted for half the protein present in the egg-white. Part of the missing protein was found by Osborne & Campbell to be in the

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* A large number of concentric rings can be seen in egg-white coagulated *in situ*, according to Remotti.

Table 12. *Composition of Sauropsid egg-white.*

Analysis of egg-white	% wet weight					% dry weight				Investigator and date
	Water	Protein	Fat	Non-nitrogenous extractives	Ash	Protein	Fat	Nitrogen	Ash	
BIRDS										
Hen	86·36	12·71	0·24	—	0·69	93·18	1·76	14·88	—	König & Krauch (1878)
,, ...	85·90	13·30	—	—	0·80	94·33	—	15·08	—	Wolff (1878)
,, ...	85·00	12·00	0·27	—	0·30	80·00	1·80	12·80	—	Bostock (1855)
,, ...	84·76	13·48	0·26	0·87	0·63	88·51	1·71	14·16	—	Stützer (1882)
,, ...	86·20	12·30	0·20	—	0·60	—	—	—	—	Langworthy (1902)
Duck	87·00	11·10	0·03	—	0·80	—	—	—	—	,,
Goose	86·30	11·60	0·02	—	0·80	—	—	—	—	,,
Turkey	86·70	11·50	0·03	—	0·80	—	—	—	—	,,
Guinea-fowl ...	86·60	11·60	0·03	—	0·80	—	—	—	—	,,
Hen	—	—	—	—	—	—	—	18·4	—	Leveque & Ponscarme (1913)
,, ...	86·61	10·13	0·14	—	0·71	81·60	1·04	—	5·27	Lebbin (1900)
,, ...	86·00	—	—	—	—	—	—	—	—	Amar (1924)
,, ...	86·72	11·21	0·46	—	0·86	84·41	3·46	—	6·48	Greshoff, Sack, van Eck & Bosz (1903)
,, ...	87·92	10·10	0·021	—	0·63	—	—	—	—	Pennington (1910)
,, ...	87·71	11·10	—	—	0·55	—	—	—	—	Kojo (1911)
Hen (av. of many different breeds)	85·49	12·25	0·20	0·87	0·87	—	—	—	—	von Czadek (1917)
,, ,,	86·23	12·00	0·59	—	0·84	—	—	—	—	Carpiaux (1903)
Hen	87·10	—	—	—	—	—	—	—	—	Davy (1862)
,, ...	86·68	12·27	—	0·38	0·66	—	—	—	—	Lehmann (1850)
,, ...	86·90	—	—	—	—	—	—	—	—	Vladimirov (1926)
,, ...	87·60	—	—	—	—	—	—	13·87	—	Aggazzotti (1919)
,, ...	85·70	12·70	0·30	0·70	0·60	—	—	—	—	Iljin (1917)
,, ...	87·70	10·80	0·14	—	0·65	—	—	—	—	van Meurs (1923)
,, ...	87·30	11·25	—	—	—	—	—	—	—	,,
,, ...	87·2	10·70	0·10	1·4	0·60	—	—	—	—	Plimmer (1921)
Duck	87·5	10·00	0·03	1·7	0·70	—	—	—	—	,,
Honey buzzard (*Pernis apivorus*)	89·25	—	—	—	—	—	—	—	—	Mörner (1912)
Pheasant (*Phasianus colchicus*)	88·36	—	—	—	—	—	—	—	—	,,
Guinea-fowl (*Meleagris gallopavo*)	88·06	—	—	—	—	—	—	—	—	,,

Goose (*Anser domesticus*)	88·86	—	—	—	—	—	—	—	—	Mörner (1912)
Duck (*Anas domesticus*)	86·68	—	—	—	—	—	—	—	—	,,
Eiderduck (*Somateria mollissima*)	87·42	—	—	—	—	—	—	—	—	,,
Cormorant (*Phalarocrocorax carbo*)	89·92	—	—	—	—	—	—	—	—	,,
Gull (*Larus canus*)	88·26	—	—	—	—	—	—	—	—	,,
Hen										
W. Leghorn ...	87·86	10·50	0·02	—	0·64	—	—	—	—	Pennington (1909)
Plym. Rock ...	87·98	10·60	0·02	—	0·63	—	—	—	—	,,
,, ...	86·51	—	—	—	—	—	—	—	—	Hartung (1902)
Nidicolous birds										
Gull	89·10	—	—	—	—	—	—	—	—	Tarchanov (1884)
Starling ...	89·90	—	—	—	—	—	—	—	—	,,
Nightingale ...	89·10	—	—	—	—	—	—	—	—	,,
Canary ...	89·00	—	—	—	—	—	—	—	—	,,
Thrush ...	90·10	—	—	—	—	—	—	—	—	,,
Raven ...	90·20	—	—	—	—	—	—	—	—	,,
Corncrake ...	89·50	—	—	—	—	—	—	—	—	,,
Dove	89·30	—	—	—	—	—	—	—	—	,,
Finch	89·40	—	—	—	—	—	—	—	—	,,
Nidifugous birds										
Plover ...	87·90	—	—	—	—	—	—	—	—	,,
Quail ...	86·60	—	—	—	—	—	—	—	—	,,
Hen	86·90	—	—	—	—	—	—	—	—	,,
Guinea-fowl ...	87·80	—	—	—	—	—	—	—	—	,,
Turkey ...	87·90	—	—	—	—	—	—	—	—	,,
Duck	88·00	—	—	—	—	—	—	—	—	,,
Goose... ...	87·60	—	—	—	—	—	—	—	—	,,
Goose	87·92	10·77	0·06	—	0·68	—	—	—	—	Hepburn & Katz (1927)
Duck	87·02	9·88	0·01	—	0·21	—	—	—	—	,, ,,
Hen	—	15·35	—	—	—	—	—	—	—	Rakusin & Flieher (1923)
REPTILE										
Marine turtle ... (*Thalassochelys corticata*)	98·0	—	—	—	—	—	—	20·0	40·0	Tomita (1929)

Table 13. *Distribution of proteins in avian egg-white*

		% of total protein in egg-white			
Species	% wet weight ovo-mucoid	ovo-mucoid	ovo-mucin	ovo-albumen	Investigator and date
Hen	—	—	7	80	Osborne & Campbell (1900)
,,	—	10·5	—	80	Komori (1926)
,,	—	7·6	—	80	Needham (1927)
,,	—	12·5	—	—	Mörner (1912)
Passeres (singing birds)					
Turdus iliacus	1·78	—	—	—	Mörner (1912)
Turdus piliaris	1·85	—	—	—	,,
Anthus pratensis	2·33	—	—	—	,,
Anthus cervinus	2·09	—	—	—	,,
Garrulus infaustus	1·56	—	—	—	,,
Corvus cornix	1·73	—	—	—	,,
Corvus monedula	1·58	—	—	—	,,
Zygodactyli (2-toed)					
Dryocopus martius	2·40	—	—	—	,,
Accipitres (birds of prey)					
Strix aluco	1·47	—	—	—	,,
Falco gyrfalco	1·53	—	—	—	,,
Falco peregrinus	1·26	—	—	—	,,
Falco aesalon	1·55	—	—	—	,,
Falco tinnunculus	1·28	—	—	—	,,
Astur palumbarius	1·45	—	—	—	,,
Buteo lagopus	2·11	—	—	—	,,
Pandion haliaëtius	1·25	—	—	—	,,
Pullastrae (doves)					
Columba livia	0·83	—	—	—	,,
Gallinae (fowls)					
Gallus domesticus	1·49	12·50	—	—	,,
Meleagris gallopavo	2·18	18·25	—	—	,,
Grallae (marsh birds)					
Charadrius apricarius	1·16	—	—	—	,,
Haematopus ostralegus	1·40	—	—	—	,,
Tringa alpina	1·05	—	—	—	,,
Phalaropus hyperboreus	1·03	—	—	—	,,
Totanus glareola	1·32	—	—	—	,,
Actitis hypoleucos	1·31	—	—	—	,,
Limosa lapponica	1·26	—	—	—	,,
Numenius arquatus	1·71	—	—	—	,,
Numenius phaeopus	1·54	—	—	—	,,
Fulica atra	1·81	—	—	—	,,
Lamellirostres (ducks)					
Anser segetum	1·57	—	—	—	,,
Fuligula marila	2·06	—	—	—	,,
Fuligula fuligula	1·45	—	—	—	,,
Oedemia fusca	1·40	—	—	—	,,
Clangula glaucion	1·56	—	—	—	,,
Somateria mollissima	2·00	15·90	—	—	
Mergus serrator	1·67	—	—	—	

Table 13 (*cont.*).

Species	% wet weight ovo-mucoid	% of total protein in egg-white: ovo-mucoid	ovo-mucin	ovo-albumen	Investigator and date
Steganopodes (pelicans)					
Phalarocrocorax carbo ...	0·21	2·08	—	—	Mörner (1912)
Phalarocrocorax graculus ...	0·46	—	—	—	,,
Longipennes (swallows)					
Larus canus	1·76	15·00	—	—	,,
Lestris crepidata	1·28	—	—	—	,,
Sterna macrura	1·36	—	—	—	,,
Sterna hirundo	1·53	—	—	—	,,
Pygopodes (divers)					
Podiceps cristatus ...	2·04	—	—	—	,,
Colymbus arcticus ...	1·88	—	—	—	,,

form of the two glucoproteins and the other albumen, while part of it was accounted for by the fact that the yield of the crystallising process is not great. Ovomucoid was originally discovered by Neumeister, who called it "pseudopeptone", and first studied by Salkovski and Zanetti.

The investigations of Osborne & Campbell, whose memoir is the best on this subject, give no very definite indication of the proportions in which these proteins make up the protein fraction of egg-white, but they put ovoalbumen at about 80 per cent., and ovomucin at about 7 (see Table 13). Later, Komori estimated that ovomucoid accounted for about 10·5 per cent. of the proteins, and in 1927 I obtained a figure of 7·6 per cent. for the same constituent. Mörner, in his extensive study of ovomucoid in numbers of birds' eggs, obtained results from which higher figures emerge on calculation, namely, from 10 to 20 per cent. The only exceptions were the pelicans, which seemed to have very little ovomucoid. The most probable relationship between the proteins is as follows: ovoalbumen 75, ovomucoid 15, ovomucin 7 and conalbumen 3 per cent., but these values are only very approximate, and further work on this point is much to be desired. Leaving out ovomucin, Wu & Ling found that the proportions were as follows (for *Gallus domesticus*): ovoalbumen 78·3, ovomucoid 12·3 and conalbumen 9·4 per cent., or 1·34, 0·211 and 0·161 gm. per cent. respectively. Certain Russian workers (Worms and Panormov) have described two proteins, anatin and anatidin, in the egg-white of the duck's egg, and three, corvin, corvinin and corvinidin, in the egg of the crow. It is not certain, however, to which of the well-known proteins of the hen's egg-white these others correspond. Judging from the percentage composition tables in Table 10 *a*, the columbin of the dove's egg corresponds to hen ovoalbumen and to duck anatinin, while duck anatin corresponds to hen ovomucin, but in the absence of definite information the question must be regarded as unsettled, and would repay further investigation.

The minimal molecular weight of ovoalbumen, according to Cohn, Hendry & Prentiss, is 33,800 (Marrack & Hewitt suggest 43,000), and its percentage composition is seen in Table 10 *a*; the best analyses are probably those of Osborne & Campbell, who give an account of its general properties. It has been further analysed by several workers who have determined the proportions of its constituent amino-acids, and whose results are seen in Table 11. The hydrolyses of Osborne, Jones & Leavenworth; Osborne & Gilbert, and of Abderhalden & Pregl were all done by acid, but those of Hugounenq & Morel and Skraup & Hummelberger were alkaline, the former using baryta. The figures agree accordingly, and all that can be said of them is that for purposes of calculation the amounts of amino-acids must be taken as minimum in each case. Attention may also be drawn to the less complete analyses of Chapman & Petrie and Hugounenq & Galimard and to the analysis of mixed egg-white proteins by Plimmer & Rosedale, using the van Slyke technique. The large amounts of hexone bases found by them contrast with those found by the remaining workers, using direct isolation, and if this is not due simply to difficulties of technique it may lead us to expect a high content of hexone bases in conalbumen and ovomucin when they come to be analysed.

In Table 10 *a* the results obtained by Gupta on the hydrolysis products of ovoalbumen are given (see also Rudd). It is noticeable in them, as in the analyses of ovoalbumen itself, that they contain a high proportion of sulphur, though not so much as ovomucoid. The spontaneous evolution of hydrogen sulphide by egg-white on standing has long been known, and was made the subject of a paper in 1893 by Rubner, Niemann & Stagnita, who found that 100 gm. of egg-white gave off when boiled with water 10·7 mgm. of H_2S. Hausmann later decided that its source must be some labile sulphydryl grouping in the ovoalbumen molecule. In 1922 Harris observed that raw egg-white was quite non-reactive towards the nitroprusside test for sulphydryl groups, but that immediately upon coagulation by heat it became vividly reactive, and gave an intense purple colour. This change only took place in conditions where denaturation of the protein was involved, and Harris suggested that this treatment might unmask a thiopeptide linkage or some similar arrangement which by hydrolysis or keto-enol transformation would give rise to an active sulphydryl group in the resulting metaprotein molecule. Later, Harris found that only 14 per cent. of the sulphur in ovoalbumen could be accounted for as cystine, so that some unknown sulphur compound must be present in considerable quantity, and an exactly similar finding was later reported by Osato for the egg-membrane protein of the herring. The cystine recoverable from serum albumen, on the other hand, accounted for 86 per cent. of the sulphur there. The possibilities of these facts with relation to the metabolism of the embryo have not yet been explored. Philothion, according to de Rey-Pailhade, exists in the egg-white of the hen but not in that of the duck.

The principal investigation of ovomucoid is that of Mörner. He had previously discovered that percaglobulin, a protein extracted from the unripe ovarial fluid of the perch (*Perca fluviatilis*) would precipitate ovomucoid from its solution. With this reagent he made an examination of a wide variety of birds' eggs, in order to study the distribution of ovomucoid. By direct estimation he found it to be present in all the eggs he studied, but it seemed to exist in two sharply distinguished forms, one which would give a precipitate with percaglobulin, and another which would not. Thus the hen (*Gallus domesticus*) with 1·46 per cent. of ovomucoid gave a highly positive reaction, but the hawk (*Astur palumbarius*) with 1·45 per cent. gave none at all. Preparations of ovomucoid from the two varieties of egg-white (see Table 10*a*) did not show up the existence of two obviously different chemical individuals, and it was concluded that the preparations were in each case mixed with a small amount of the other substance. Moreover, of the egg-whites which gave a positive reaction with percaglobulin, some contained ovomucoid precipitable with Esbach's reagent (e.g. *Clangula glaucion* and *Somateria mollissima*) and others contained an ovomucoid which could not be so precipitated (e.g. *Gallus domesticus* and *Podiceps cristatus*). It is quite uncertain how many of the effects observed by Mörner are due to physical and colloidal rather than to chemical differences, and the whole question should be reinvestigated. There seemed to be no special significance in the distribution of the ovomucoid which was precipitable by percaglobulin; thus it was present in the accipitres, grallae, lamellirostres, longipennes and pygopodes, but not in the passeres, zygodactyli, pullastrae and steganopodes. As for the fowls, it was present in the eggs of the hen and pheasant, but not in those of the guinea-fowl. Mörner was inclined to agree with Milesi's view that ovomucoid did not exist as such in the natural egg-white at all.

Table 14. *Variations in properties of avian egg-white.*

		Consistency and colour of coagulum	Coagulation point of the egg-white in degrees Fahrenheit
Nidifugous	Hen	Hard white	160
Nidicolous	Pigeon	Pretty firm, bluish ...	189
	Jay	Soft, white, translucent ...	184
	Missel thrush ...	Soft, transparent ...	188
	Starling	Firm white	195
	Robin	Soft white	187
	Hedge-sparrow ...	Pretty firm, greenish, transparent	168
	Golden-crested wren	Soft, bluish, semi-transparent	—

As has already been observed, Sir Thomas Browne was one of the first to note that the coagulated egg-white of the gull's egg was quite different in consistency and translucency from that of the hen's egg. In 1863 Davy collected some data on these points, which are shown in Table 14, and Tarchanov devoted much time to the question in the 'eighties of the last century. He found that the whites of many kinds of eggs would not coagulate in the ordinary way on boiling, but either remained liquid and transparent or else set to a watery translucent jelly. This he called "tataeiweiss", and as he went on to examine the distribution of this property he found that it was associated with the hatching quality of the bird in question. Thus all nidifugous birds, whose chicks are born fully feathered ("downy") and soon leave the nest, had eggs with ordinary egg-white, but all nidicolous ones, whose chicks are hatched as "squabs" or naked and weak, and have some development yet to complete, had eggs with uncoagulable or transparent egg-white. Thus the sand-martin, linnet, finch, thrush, canary, crow, dove, rook, nightingale, robin, starling (roughly passeres and pullastrae), all had *tataeiweiss*; while the hen, duck, goose, guinea-fowl, partridge and corncrake had ordinary white. This classification agreed roughly with Davy's high and low coagulation points for the egg-white, and corresponded on the whole to Mörner's two classes, the former having ovomucoid not precipitable with percaglobulin and the latter having the precipitable variety, but to this there were some exceptions; thus the plover's ovomucoid could be so precipitated, but its egg-white was *tata* and it yet produced fully-feathered chicks. It was, however, the only exception to Tarchanov's generalisation. (It should be explained that the word *tata* was derived from the name of Tarchanov's small daughter.) Tarchanov found that *tata* egg-white was about 3 per cent. richer in water than the other kind, a conclusion which Mörner's later analyses did not confirm. He also said that it was alkaline to litmus, but became less so as the *tata* eggs developed. This agrees with the later classical work of Aggazzotti on the reaction of the egg-white of the hen's egg during its development. Tarchanov reported that *tata* egg-white could be made to coagulate at ordinary temperatures by the addition of a little potassium sulphate, and that it would itself coagulate if the temperature was raised well above the boiling-point of water. It was, he said, more easily digested by enzymes, it putrefied more readily, and during development it changed into a form resembling ordinary egg-white. He made some studies on its secretion by the oviduct of these birds, and was the first to perform the experiment of putting a ball (in his case a lump of amber) at the top of the oviduct and seeing it emerge at the bottom with layers of egg-white and a shell secreted around it. The change during development from *tata* to ordinary egg-white Tarchanov found he could imitate by bubbling carbon dioxide through the original white, after which it would coagulate in the usual way. On the other hand, he found that if he soaked normal hen's eggs in a 10 per cent. solution of alkali the white took on the properties of *tata* egg-white, and became just like the glassy material in the sand-martin's egg. He suggested some relation between these phenomena and the alkali-albuminate of Lieberkühn, but did little to determine its chemical relationships. He was unable to get any development in the case of hen's eggs soaked in alkali.

In 1891 Zoth took up the whole question of *tataeiweiss* once more. He was led to do so on account of some researches which he had been making on the effect produced on serum-clotting by various concentrations of alkali, and which showed that the clot could vary very greatly in its properties, from opacity to almost perfect transparency, for instance. Tarchanov had decided that the transparent coagulum of the nidicolous egg-whites was not to be identified with that produced by sodium or potassium albuminate, but Zoth succeeded in showing that the differences were not sufficient to distinguish them. Zoth fully confirmed Tarchanov's finding that ordinary egg-white could be made to pass over into nidicolous egg-white by treating it with 10 per cent. potash in the cold for ten days, and was able to explain all the differences between *tataeiweiss* and alkali ovoalbuminate as due to variations in the amount of alkali present, or rather the amount of cation as compared to anion. It is most unfortunate that we have no detailed ash analyses of the egg-whites of nidicolous birds, for, as will later be seen, the egg-white of the hen has rather more total anion than total cation, and this relationship might be expected to be even more strongly marked in the case of nidicolous egg-white, perhaps, indeed, as much as to counterbalance the excess of cation over anion in the yolk. There can be no doubt, however, that the egg-whites of nidicolous birds are relatively richer in alkali than are those of others, and it is this, combined with their different water and total ash content, which causes the albumen to coagulate differently from those of others. Thus, if 5 c.c. of filtered egg-white from a fresh hen's egg be put in each of three small Erlenmeyer flasks, 2 c.c. of water added to *A*, 2 c.c. of 0·89 per cent. KOH to *B*, and 2 c.c. of a mixture of equal parts 0·89 per cent. KOH and 0·66 per cent. NaCl to *C*, the coagulum in *A* will be the usual white, thick, solid and opaque mass, while the other two will be transparent like *tataeiweiss*, slightly opalescent, more or less liquid, and *C* more opalescent than *B*. It would be interesting to reinvestigate the whole question anew in the light of recent knowledge and technique.

Another curious effect was noted by Melsens and Gautier. Melsens found that, if a stream of carbon dioxide, hydrogen, nitrogen or oxygen, was passed through dilute egg-white, or if it was shaken violently, a precipitate of fibrous membranous shreds was formed. Gautier observed that about 1·5 per cent. of the protein was thus changed; he filtered it off and determined its elementary composition, which showed nothing remarkable. He concluded that a protein which he called "ovofibrinogen" existed in the egg-white, and even suggested that an "ovothrombin" was present to turn it into "ovofibrin". He apparently thought that the ovofibrin was incorporated without change into the substance of the embryo. The subject has not received any attention since the time of Gautier, but it is probable that this phenomenon is explained by the work of Young; Dreyer & Hanssen and others, on the high instability of protein solutions.

Peptones were reported by Reichert to exist in fresh egg-white.

Wu & Ling have recently studied the coagulation of ovoalbumen by strong mechanical agitation. The fact that conalbumen is not coagulable by such means gave them a method of estimating it in egg-white. Thus they obtained the following figures for *Gallus domesticus* egg-white:

	Nitrogen
Total N (ovoalbumen + conalbumen + ovomucoid)	1·71 gm. %
After shaking (conalbumen + ovomucoid) ...	0·372 ,,
After shaking and heating (ovomucoid) ...	0·211 ,,

Coagulation of ovoalbumen by shaking was not separable into two stages (denaturation and agglutination) like that by heat or alcohol. The isoelectric point of the protein was the most favourable for shaking coagulation (pH 4·8) and the Q_{10} of the reaction was 1·9. Piettre has published a method for separating the proteins which involves the use of acetone.

The relationships between the avian egg-white proteins have been the subject of some interesting immunological work. The earliest investigators who crystallised ovoalbumen found that perfect freshness was necessary, for at room temperature the crystallisable protein gradually turns into a non-crystallisable one. Bidault & Blaignan found that this process could be arrested by placing the egg at 0°. Sørensen & Høyrup suggested that the protein formed was conalbumen and wished to look upon the latter as a product of ovoalbumen. Hektoen & Cole, however, first showed that though ovoalbumen was distinct from the serum albumen of the hen immunologically, conalbumen was not, and then went on to demonstrate that during the loss of crystallisable ovoalbumen which takes place as the egg ages, there was no corresponding increase in conalbumen. We must therefore look upon the latter as probably identical with the serum albumen of the adult: and perhaps only present in the egg owing to the inefficiency of the oviduct.

The analyses of ovomucoid and Eichholz's ovomucin, as well as the fragmentary one of conalbumen, will be found in Tables 10 *a* and 11. Willanen found that ovomucoid was much more susceptible to hydrolysis by pepsin than by trypsin (see later under enzymes and antitrypsin). For its properties see the papers of Mörner and Neumann. Both the glucoproteins have twice as much sulphur as ovoalbumen. Their carbohydrate content has been the subject of a great amount of discussion and experimental work. Berzelius was the first to draw attention to certain similarities between the breakdown-products of sugars and proteins when acted upon by boiling acids. In 1876 Schützenberger asserted that the ovoalbumen molecule contained a carbohydrate group, basing his views on positive results with Trommer's test after total hydrolysis. In later years a number of workers supported the view that the carbohydrate was glucose, using in different cases methods of varying reliability, e.g. Krukenberg in 1885, Hofmeister and Kravkov in 1897, and Blumenthal; Blumenthal & Mayer and Mayer in 1898 and 1899. Spencer and Mörner, however, failed to get any evidence of a carbohydrate group after hydrolysis, and reported their negative results in 1898. Weiss, about the same time, thought he could identify a methyl pentose among the hydrolysis products, but he was never confirmed. Seemann was the first to announce that the carbohydrate was glucosamine, and his discovery was quickly confirmed by Frankel and Langstein. These later workers began to attempt quantitative estimation of the sugar, and their figures are given in Table 15. Pavy, using the then recently discovered osazone technique, made a study of a variety of proteins, and showed, as might be expected, that the yield from ovoalbumen was always greatly less than from ovomucoid. Eichholz obtained glucosazone from ovoalbumen, ovomucoid and ovomucin, but not from either serum albumen or casein. On the whole, it is most likely that ovoalbumen contains extremely little glucosamine, and the figures recorded in the literature for this are probably due to contamination with ovomucin. This is the view of Osborne, Jones & Leavenworth, for neither they nor Osborne & Campbell obtained any glucosamine from their very carefully purified ovoalbumen. Komori has prepared from ovomucoid, and Frankel & Jellinek from ovoalbumen, polysaccharide-like substances which they regard as the prosthetic group containing all the glucose. Following this up Levene & Mori have prepared a trisaccharide containing glucosamine and mannose from egg-white. Ovoalbumen contains 0·26 % of this substance, coagulated egg-white 1·9 %, and ovomucoid 5·1 %. According to Levene & Rothen the molecule consists of four trisaccharides each containing one molecule of glucosamine and two of mannose.

Table 15. *Ovoalbumen and ovomucoid glucosamine content.*

	Ovoalbumen %	Ovomucoid %
Hen (*Gallus domesticus*)		
Hofmeister (1898)	—	15·0
Seemann (1898)	9·0	34·9
Langstein (1900)	10·5	—
Willanen (1906)	—	19·5–22·3
Pavy (1907)	—	21·7
Samuely (1911)	—	29·4
Neuberg & Schewket (1912)	—	24·0
Zeller (1913)	—	33·7
Needham (1927)	—	11·5
Abderhalden, Bergell & Dorpinghaus (1904)	Trace	—
Neuberg (1901)	,,	—
Blumenthal & Mayer (1900)	,,	—
Izumi (1924)	3·4	—
Tillmans & Philippi (1929)	3·7	7·4
Turtle (*Thalassochelys corticata*)		
Takahashi (1929)	—	6·2

		Glucose % by hydrolysis with	
		10 % KOH	5 % HCl
Bywaters (1909)	Seromucoid	12·0	15·9
Pavy (1899)	Ovomucoid	12·3	21·7
	Osseomucoid	4·6	10·8
	Tendon mucoid	3·3	11·6
	Ovoalbumen	2·6	2·5
	Serum globulin	2·8	2·8

The free carbohydrate of the egg-white has also received a great amount of attention from an early date. In 1846 Winckler isolated a quantity of a sugar (4 grains) from the egg-white of the hen's egg, and identified it as lactose. "Physiologists", he said, "will be able to tell me whether this is of importance for the embryo or whether it was some abnormality." The observation has not since been repeated, and it is in the highest degree unlikely that any lactose was ever in an egg, unless the diet of the hen was a very unusual one. Budge and Aldridge soon were at work on this subject, the former concluding that the carbohydrate was glucose, but suggesting that it might form a disaccharide during development, and the latter making no concession to Winckler. The presence of glucose was afterwards abundantly established by the work of Barreswil; Lehmann; Meissner; Salkovski; and Pavy. Later many quantitative estimations were made, and these are collected together in Table 16. The older figures for free carbohydrate may be regarded as fairly

Table 16. *Carbohydrate distribution in avian egg.*

Percentages of wet weights are indicated by italic figures; mgm. per whole egg, per white, or per yolk by ordinary figures.

Species	Whole egg				Egg-white			Egg-yolk				Investigator, date and method
	Total glucose	Free glucose	Gly-cogen	Total combined glucose	Free glucose	Gly-cogen	Total combined glucose	Total glucose	Free glucose	Gly-cogen	Total combined glucose	
Hen	—	40–97	—	—	—	—	—	—	—	—	—	Müller & Masayama (1900) (?)
,,	335	—	—	—	—	—	—	—	—	—	—	Needham (1927) (Hagedorn-Jensen)
,,	*670*	—	—	—	—	—	—	—	—	—	—	
,,	—	184	—	—	—	—	—	—	—	—	—	Idzumi (1924) (Momose-Pavy)
,,	—	*350*	—	—	*280*	—	—	—	*60*	—	—	,, ,,
,,	507	216	3·15	285	—	—	—	—	—	—	—	Sakuragi (1917) (Momose-Pavy)
,,	*1014*	*443*	*6·3*	*571*	*496*	*3·2*	—	—	*333*	*2·2*	—	,, ,,
,,	—	—	—	—	*330*	—	—	—	—	—	—	Pavy (1894) (Pavy)
,,	—	—	—	—	106	—	—	—	—	—	—	Bywaters (1913) (Pavy)
,,	—	—	—	—	*320*	—	—	—	—	—	—	,, ,,
,,	—	193	—	—	—	—	—	—	—	—	—	Satô (1916) (Schenk-Bertrand)
,,	—	—	—	—	*470*	—	—	—	*220*	—	—	,, ,,
,,	—	183	—	—	*470*	—	—	—	*240*	—	—	Tomita (1921) (Schenk-Bertrand)
,,	—	*370*	—	—	—	—	—	—	—	—	—	Dastre (?) (?)
,,	—	221	—	—	—	—	—	—	—	—	—	Gadaskina (1926) (Galwialo)
,,	—	—	—	—	*400–500*	—	—	—	*300*	—	—	Roger (1908) (?)
,,	—	173	—	—	—	—	—	—	—	—	—	Vladimirov & Schmidt (1926) (Hagedorn-Jensen)
,,	—	*439*	—	—	—	—	—	—	*199*	—	—	
,,	—	156	—	—	—	—	—	—	—	—	—	Pennington, Hendrickson, Conolly & Hendrix (1915) (?)
,,	—	—	—	—	*440*	—	—	—	*180*	—	—	
,,	—	231	—	—	—	—	—	—	—	—	—	Hepburn & St John (1921) (Folin & Wu)
,,	—	*450*	—	—	*470*	—	—	—	*140*	—	—	
,,	—	228	—	—	—	—	—	—	135	—	—	Kojo (1911) (Fehling and fermentation)
,,	—	—	—	—	*550*	—	—	—	*272*	—	—	
,,	—	—	—	—	*800*	—	—	—	—	—	—	Winckler (1846) (as lactose, recalc. by Mörner)
,,	—	—	—	—	128	—	—	—	—	—	—	Mörner (1912) (Fehling)
,,	—	—	—	—	*400*	—	—	—	—	—	—	,, ,,
,,	—	—	—	—	*60*	—	—	—	—	—	—	Lehmann (1850) (CO_2 given off by fermentation)
,,	—	*360*	—	—	—	—	—	—	—	—	—	Bierry, Hazard & Ranc (1912) (?)
,,	—	—	—	—	*1000*	—	—	—	—	—	—	Meissner (1867) (Fehling)
,,	—	—	—	—	*750*	—	—	—	—	—	—	Gautier (1904) (?)

Hen	—	—	—	—	*120*	—	—	—	—	—	—	Salkovski (1893) (polarimetr.)
,,	—	—	—	—	*237*	—	—	*312*	*55*	—	*55*	Diamare (1909 and 1910) (?)
Goose	*400*	—	—	—	*520*	—	—	—	*120*	—	—	Hepburn & Katz (1927) (?)
Duck	*180*	—	—	—	*550*	—	—	—	*230*	—	—	,, ,,
Hen	—	—	—	—	*464**	—	*221*	—	*245†*	—	*118*	Pucher (1928) (Folin)
,,	—	—	—	—	—	—	*405‡*	—	—	—	*297‡*	,, (Benedict)

* 99·2 % of this is fermentable. † 90 % of this is fermentable. ‡ 50 % of this is fermentable.

Species	Free glucose in egg-white	Investigator and date
Passeres (singing birds)		
Turdus iliacus ...	*290*	Mörner (1912) (Fehling & polarimetr.)
Turdus piliaris ...	*270*	,, ,,
Anthus pratensis ...	*320*	,, ,,
Anthus cervinus ...	*300*	,, ,,
Garrulus infaustus ...	*290*	,, ,,
Corvus cornix ...	*270*	,, ,,
Corvus monedula ...	*280*	,, ,,
Zygodactyli (2-toed)		
Dryocopus martius ...	*230*	,, ,,
Accipitres (birds of prey)		
Strix aluco ...	*180*	,, ,,
Falco gyrfalco ...	*230*	,, ,,
Falco peregrinus ...	*160*	,, ,,
Falco aesalon ...	*160*	,, ,,
Falco tinnunculus ...	*260*	,, ,,
Astur palumbarius ...	*190*	,, ,,
Buteo lagopus ...	*160*	,, ,,
Pandion haliaëtius ...	*240*	,, ,,
Pullastrae (doves)		
Columba livia ...	*320*	,, ,,
Gallinae (fowls)		
Gallus domesticus ...	*400*	,, ,,
Meleagris gallopavo	*180*	,, ,,
Grallae (marsh birds)		
Charadrius apricarius	*290*	,, ,,
Haematopus ostralegus	*200*	,, ,,
Tringa alpina ...	*200*	,, ,,

Species	Free glucose in egg-white	Investigator and date
Grallae (marsh birds)		
Phalaropus hyperboreus ...	*200*	Mörner (1912) (Fehling & polarimetr.)
Totanus glareola ...	*120*	,, ,,
Actitis hypoleucos ...	*130*	,, ,,
Limosa lapponica ...	*190*	,, ,,
Numenius arquatus ...	*170*	,, ,,
Numenius phaeopus ...	*230*	,, ,,
Fulica atra	*190*	,, ,,
Lamellirostres (ducks)		
Anser segetum	*260*	,, ,,
Fuligula marila... ...	*220*	,, ,,
Fuligula fuligula ...	*190*	,, ,,
Oedemia fusca	*150*	,, ,,
Clangula glaucion ...	*260*	,, ,,
Somateria mollissima ...	*290*	,, ,,
Mergus serrator ...	*290*	,, ,,
Steganopodes (pelicans)		
Phalarocrocorax carbo ...	*240*	,, ,,
Phalarocrocorax graculus	*150*	,,
Longipennes (swallows)		
Larus canus	*200*	,, ,,
Lestris crepidata ...	*160*	,, ,,
Sterna macrura	*130*	,, ,,
Sterna hirundo	*180*	,, ,,
Pygopodes (divers)		
Podiceps cristatus ...	*240*	,, ,,
Colymbus arcticus ...	*130*	,, ,,

trustworthy, but not for combined sugar, in view of the demonstration of Holden that all copper-reducing methods are seriously interfered with by the presence of amino-acids and protein breakdown products. No method at present in use gives satisfactory results in those conditions, but the most reliable is that of Hagedorn & Jensen. No estimations of total carbohydrate in egg-white alone at present exist, but there is a single figure for glycogen due to Sakuragi. Mörner found no evidence of fructose, pentoses or maltose.

A curious phenomenon: the fluorescence of egg-white has been reported by van Waegeningh & Heesterman, but it only occurs if the egg is not perfectly fresh, and is therefore probably not physiological.

1·7. The Avian Yolk

The vitelline membrane was investigated by Liebermann in 1888, who found that it consisted almost exclusively of keratin. This he purified, and, having freed it from ash, made an elementary analysis of it, which is shown in Table 10 *a*. Some experiments which demonstrate the peculiarities of the vitelline membrane have been devised by Osborne & Kincaid. They found that a fresh yolk floated into distilled water, 0·9 per cent. NaCl solution, or glycerol, behaved exactly like a red blood corpuscle in that it swelled up and burst in the former, and shrank to a corrugated globe in the latter, while in the isotonic salt solution it remained unchanged. But with other treatment, nothing took place which corresponded to haemolysis. If the yolk was put into 10 per cent. NaCl solution, it did not shrink, as had been expected, but swelled up, owing to the penetration of the saline and the consequent osmotic pressure due to the dissolving of the vitellin in the saline. This showed at once the scleroprotein nature of the membrane and its impermeability to vitellin even when in solution. The membrane is also impermeable to phosphatides and fats dissolved in ether, for if a yolk is put into ether it sinks and swells, so that the upper pole is distended by an accumulation of deeply pigmented ether. But until the yolk bursts, as it eventually does, not a trace of pigment or other substance passes out into the ether, and the same results were found with chloroform and carbon disulphide. In alcohol, on the other hand, there is no swelling, for the alcoholic solution of phosphatides and other bodies can pass out through the keratin membrane. It would be very interesting to make a more extended study of the osmotic properties of the vitelline membrane (see in this connection Section 5·6).

The yolk of the egg was investigated earlier in the modern period than the white. We may pass directly, excluding the curious analysis of the eggs of *Struthio casuarius* by Holger in 1822, to the papers of Gobley, which appeared from 1846 to 1850, and which, with those of Valenciennes & Frémy from 1854 to 1856, still remain models of embryo-chemical work. "John, a German chemist," said Gobley, "appears to have been the first to occupy himself with serious researches on the yolk of the egg. The chemists who preceded him considered it as made up only of water, albumen, oil, gelatine, and colouring matter; such was the opinion of Macquer, Fourcroy, and Thomson. John concluded from his experiments, which he published in 1811, that the yolk was composed of water, a sweet yellow oil, traces of a free acid which he thought was phosphoric acid, and a small amount of a brownish red substance, soluble in ether and alcohol. Besides these he found gelatine, sulphur, and a great deal of a modified albuminous substance." Gobley referred also to the work of Prout, of Chevreul, of Berzelius and of Lecanu, who discovered the presence of cholesterol in yolk in 1829.

Gobley himself found in the yolk nearly all the substances which we now know to be there. His own list of them ran as follows:

1. Water.
2. An albuminous matter, "vitellin".
3. Olein.
4. Margarin.
5. Cholesterin.
6. Margaric acid.
7. Oleic acid.
8. Phosphoglycerilic acid.
9. Lactic acid.
10. Salts such as chloride of sodium, chloride of potassium, chlorhydrate of ammonium, sulphate of potash, phosphate of lime, and phosphate of magnesia.
11. A yellow colouring matter and a red colouring matter.
12. An organic substance containing nitrogen, but which is not albuminous.

Most of the constituents of egg-yolk may be recognised under this

Table 17. *Composition of Sauropsid egg-yolk.*

Birds	% wet weight						% dry weight						Investigator and date
	Water	Protein	Fat	Non-nitrogenous extractives	Ash	Lipoids	Protein	Fat	Nitrogen	Ash	Non-nitrogenous extractives	Lipoids	
Dwarf hen ...	52·67	19·49	27·84	—	—	—	—	—	—	—	—	—	Sacc (1847)
Hen	—	—	—	—	—	—	—	48·24	—	—	—	—	Jeans (1903)
„	51·00	—	—	—	—	—	33·00	64·00	—	2·5	—	—	Laves & Grohmann (1903)
„	51·48	15·76	31·43	0·40	1·33	8·43	32·48	64·82	5·19	—	—	—	Gobley (1847)
„	47·19	15·63	36·21	—	0·97	17·42	29·60	68·57	4·73	—	—	—	Parke (1868)
„	50·84	16·12	30·54	0·94	1·55	—	32·79	62·13	5·25	—	—	—	König & Krauch (1878)
„	53·78	17·48	28·75	—	0·53	—	37·80	62·20	6·03	—	—	—	Prout (1859)
„	51·85	15·62	30·00	0·88	1·65	—	32·44	62·31	5·19	—	—	—	Stützer (1882)
„	49·5	15·7	33·3	—	1·1	—	28·8	69·20	—	2·3	0·3	—	Langworthy (1901)
Duck	45·8	16·8	36·2	—	1·2	—	31·0	66·80	—	2·0	0·3	—	„
Goose	44·1	17·3	36·2	—	1·3	—	31·0	64·30	—	2·3	1·3	—	„
Turkey	48·3	17·4	32·9	—	1·2	—	33·6	63·60	—	2·3	0·8	—	„
Guinea-fowl ...	49·7	16·7	31·8	—	1·2	—	33·2	63·20	—	2·4	0·8	—	„
Hen	47·53	17·45	33·32	—	1·67	—	33·25	63·51	—	3·18	—	—	Lebbin (1900)
Hen (W. Leghorn)	47·44	17·44	32·65	—	1·90	—	—	—	—	—	—	—	Pennington (1910)
„ (Plym. Rock)	47·84	16·75	32·70	—	1·77	—	—	—	—	—	—	—	„
Hen	49·73	16·66	—	—	1·44	—	—	—	—	—	—	—	Kojo (1911)
„	51·12	—	25·86	—	1·68	—	—	—	—	—	—	—	Juckenack & Posternak (1904)
„	—	—	—	—	—	9·41	—	—	—	—	—	—	Manasse (1906)
„	—	—	—	—	—	9·50	—	—	—	—	—	—	Roaf & Edie (1905)
Hen (av. of many different breeds)	49·19	16·68	31·17	1·39	1·57	—	—	—	—	—	—	—	von Czadek (1917)
„ „	51·55	15·61	30·91	—	—	7·19	—	—	—	—	—	—	Carpiaux (1903)
Hen	47·10	15·5	33·3	2·1	2·0	—	—	—	—	—	—	—	Plimmer (1921)
Duck	45·10	15·3	36·5	1·6	1·5	—	—	—	—	—	—	—	„
Hen	—	17·50	29·80	—	—	—	—	—	—	—	—	—	Malcolm (1902)
„	—	—	21·74	—	—	11·57	—	—	—	—	—	—	Serono & Palozzi (1911)
„	47·06	—	—	—	—	—	30·98	—	—	2·22	—	—	Cook (1908)
„	47·80	—	—	—	—	—	28·80	49·20	—	—	—	20·9	Riddle (1911)
„	47·35	—	—	—	—	—	—	—	—	—	—	—	Greenlee (1911)

Hen (yellow yolk)*	45·40	15·04	25·25	0·36	0·44	11·15	28·36	49·51	—	0·85	0·70	20·58	Spohn & Riddle (1916)
,, (white yolk)*	86·66	4·60	2·39	0·40	0·62	1·13	44·44	23·11	—	6·03	3·88	10·91	,,
Jungle fowl ...	48·71	15·27	23·96	1·04	0·82	10·21	29·78	46·70	—	1·60	2·03	19·90	,,
Pigeon	55·56	12·89	20·81	0·89	0·94	9·11	29·00	47·00	—	2·11	2·00	20·50	,,
Hen	—	—	—	—	—	—	—	—	—	—	—	15·35	Mesernitzki (1907)
,, ...	49·90	—	—	—	—	—	—	—	—	—	—	—	Vladimirov (1926)
,, ...	50·80	16·20	31·70	0·10	1·10	8·10	—	—	—	—	—	—	Iljin (1917)
,, ...	49·55	16·65	30·80	—	1·40	—	—	—	—	—	—	—	van Meurs (1923)
,, ...	49·70	16·90	30·55	—	—	—	—	—	—	—	—	—	,,
Nidicolous birds													
Starling ...	56·60	—	—	—	—	—	—	—	—	—	—	—	Tarchanov (1884)
Raven	57·10	—	—	—	—	—	—	—	—	—	—	—	,,
Corncrake ...	58·00	—	—	—	—	—	—	—	—	—	—	—	,,
Dove	57·90	—	—	—	—	—	—	—	—	—	—	—	,,
Nidifugous birds													
Plover	50·30	—	—	—	—	—	—	—	—	—	—	—	,,
Quail	41·80	—	—	—	—	—	—	—	—	—	—	—	,,
Hen	49·80	—	—	—	—	—	—	—	—	—	—	—	,,
Guinea-fowl ...	48·70	—	—	—	—	—	—	—	—	—	—	—	,,
Hen	49·42	—	—	—	—	—	—	—	—	—	—	—	Hartung (1902)
,, ...	—	—	32·8	—	—	9·39	—	—	—	—	—	—	McCollum, Halpin & Drescher (1912)
Pigeon	54·31	—	31·00	—	—	—	—	—	—	—	—	23·90	Glikin (1908)
Turtle-dove ...	82·05(?)	—	—	—	—	—	—	—	—	—	—	20·55	,,
Hen	45·69	—	—	—	—	7·4	—	—	—	—	—	13·71	,,
,, ...	51·0	—	32·50	—	—	3·0	—	—	—	—	—	—	Bergell (1900)
Duck	44·87	—	38·87	—	—	—	—	—	—	—	—	—	Vignon & Meunier (1904)
Goose	41·1	18·67	35·74	—	1·73	—	—	—	—	—	—	—	Hepburn & Katz (1927)
Duck	43·69	18·42	35·04	—	1·35	—	—	—	—	—	—	—	,, ,,
Hen (Plym. Rock)	48·03	16·75	32·70	—	—	—	—	—	—	—	—	—	Hepburn, Jenkins, Swan & Sherwood (1920)
Hen (W. Leghorn)	47·43	17·44	32·65	—	—	—	—	—	—	—	—	—	,, ,,
Hen	48·00	—	—	—	—	—	—	—	—	—	—	—	Amar (1924)
REPTILE													
Marine turtle (*Thalassochelys corticata*)	72·5	—	—	15·0	—	—	—	32·0	10·7	4·3	—	—	Tomita (1929)

* Averages of White Wyandotte and Plymouth Rock.

old-fashioned terminology. Gobley made many quantitative observations on the various substances, and his figures are given in Table 17, which sums up all the analyses that have been made of the yolk in the eggs of birds. The original discovery of vitellin was made by Dumas & Cahours, but Gobley was the first to make an extended study of it. His elementary analysis is given in Table 10 *a*. He knew that it contained both sulphur and phosphorus. Gobley was able to isolate oleic and margaric (palmitic) acids from the fat fraction of the yolk, but, unlike Planche twenty-five years before, he got no stearic, and Kodweiss, one year later, reported its presence under the impression that it had not been found before. Gobley, however, was easily able to repeat Lecanu's discovery of the presence of cholesterol, and made a remarkable examination of the lipoids. "These viscous materials", he said, "appear to have been considered by John as not being of a fatty nature at all. They form the most interesting part of the yolk; they contain all the phosphorus which exists there in considerable quantity." He analysed the glycerophosphoric acid which he obtained from the lipoid, which he named "lecithin", and speculated as to the significance which it might have for the growth of the embryo. He also recognised that fatty acids and nitrogen were present in the viscous matter.

Ten years later Valenciennes & Frémy made a further examination of the yolks of a large variety of eggs with special reference to vitellin. It was they who discovered substances very similar to vitellin in the eggs of reptiles and fishes; these they named the ichthulins. As regards the eggs of birds, they contented themselves with confirming the results of the previous investigators, but they regarded vitellin as having practically the same constitution as fibrin, on the grounds of elementary composition only. At the same time, they held it to be a different compound because it would not, like blood fibrin, decompose hydrogen peroxide.

If Table 17 is examined, it will be seen that the yolk is much drier than the white in all birds' eggs examined, having only about 50 per cent. of water as against the 85 per cent. of the latter. On the other hand, the percentage of fatty substances and lipoids is much higher, being just about double the amount of protein, whether related to wet weight or to dry. It is noticeable from the analyses of Tarchanov that the yolks of eggs from nidicolous birds having a short incubation time are about 10 per cent. richer in water than yolks from the eggs of nidifugous birds. This must imply that the greater requirement for nutrient material in the latter case has, as it were, packed the fat tighter into the yolk. Exactly the same relationship is brought out from the figures of Spohn & Riddle, who compared the pigeon which hatches out as a squab with the hen which hatches out as a fully-feathered chick. Spohn & Riddle's analyses are the only complete ones we have for a nidicolous egg, and bear clearly the same relationship, for there is less protein and less fat, relatively, in the pigeon's egg than in the hen's. The ash content and the amount of non-nitrogenous extractive substances seem, however, to be slightly higher in the latter case. Langworthy's figures were all obtained from the eggs of nidifugous birds, and they show a great similarity among themselves. More delicate consideration, of course, reveals differences according to breed in the hen's egg, e.g. the figures of Pennington and his collaborators, but these are of a comparatively minor order.

The most interesting analyses are those of Spohn & Riddle. They compared the egg of the jungle-fowl, which is supposed to have been the evolutionary ancestor of the domestic hen, with averaged figures for hen's eggs of various breeds, and, as is evident, there was a very close agreement. They also analysed the white yolk as distinct from the yellow yolk of the hen's egg. When the yolk begins to be formed in the ovary of the hen, it is white and not yellow, and not until the critical point in its maturation is reached, when its growth-rate completely changes, does it begin to store lipochrome pigment. This change in growth-rate, which has been observed by other workers as well as Riddle (e.g. Walton), will be dealt with in more detail in the appendix on maturation. Von Hemsbach, in a paper on the milky or white yolk of the birds, in 1851, suggested that the *corpus luteum* of mammals corresponded to the yellow yolk of birds, and that the mammalian ovum having been shed out of the ovary into the Fallopian tube and uterus, the fats and lipochrome pigment were laid down in the Graafian follicle instead of around the white "ovum". Von Hemsbach also supported the view already mentioned that the shells of avian and amphibian eggs corresponded to the decidua of mammals. He laid stress on the work of Zwicky and Göbel, who had investigated the pigments of yolk and *corpus luteum*, and had thought them to be identical. This subject will be referred to again under the head of pigments.

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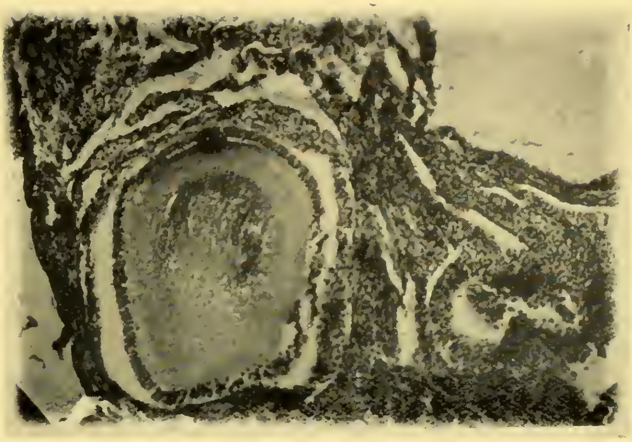
In the fresh egg, as laid, the white yolk occupies a central position, and is surrounded by concentric layers of yellow yolk. But as a kind of cylindrical prolongation of the white yolk reaches to the surface of the vitellus underneath the blastodisc or germinal spot, the white yolk must be considered the first food of the embryo, and, until its composition was determined, it was not possible to say what sort of nutrient environment the embryo possessed in the very early days of development, although the composition of the yellow yolk would give this for the later period. The histological differences between white and yellow yolk had been known for a long time (see Purkinje; His; Leuckart; Klebs; Dursy; Stricker; and Virchow) but Riddle and Spohn & Riddle were the first to approach the subject chemically. Their figures showed that the white yolk much the more nearly approximated to the contents of invertebrate eggs with holoblastic cleavage, and living undifferentiated tissue generally. Instead of 45 per cent. of water, the white yolk had 86 per cent., instead of 15 per cent. of protein, it had only 4, and instead of 25 per cent. of fat it had only 2. Thus in its water-content, it was much more like (*a*) egg-white and (*b*) the young embryo itself than like ordinary yolk, while instead of having twice as much fat as protein it had twice as much protein as fat. These data are extremely interesting in view of the facts that are known about the sources of energy made use of by the embryo during its development. Although by far the greatest proportion by weight of substance combusted during embryonic life is fat, yet, in the early stages, the embryo undoubtedly gets its energy preponderantly from protein and carbohydrate (see the whole of Section 7). The percentage of non-nitrogenous extractives did not differ much between white and yellow yolk in the experiments of Spohn & Riddle, but it would be very interesting to know the relative amounts of carbohydrate, and analyses to discover this should certainly be done. Again, the yellow yolk contained eight times less ash than the white yolk, a finding which acquires considerable significance from the fact that, if the ratio inorganic substance/organic substance in the embryonic body is plotted, it is seen to descend steadily from the beginning of development (see Fig. 249). Moreover, as Mendeléef has shown, early embryonic cells contain twice as much electrolyte as those of later stages (see Section 5·8). The amount of phosphatide in the yellow yolk, furthermore, was ten times that in the white, a significant difference; for, as Plimmer & Scott have shown, one of the main functions of the phosphatide is in furnishing phosphorus for the embryonic bones during the period of ossification, a requirement which is not present in the earlier stages of the development. The histochemical work of Marza, who compared the white and yellow yolk following the method of Romieu, is in agreement with this, for he found the elements of the yellow yolk to be richer than those of the white. (See Plate X.)

1·8. The Avian Yolk-proteins

As regards the protein, vitellin (Tables 10*a* and 11), several interesting points are to be observed. The best elementary analyses of ovovitellin are probably those of Osborne & Campbell. After its discovery by Dumas & Cahours, Gobley, and Valenciennes & Frémy, it was studied by Hoppe-Seyler, and now for the first time with special reference to its position in the classification of the proteins. Virchow had some time before then suggested that the yolk-platelets, familiar to histologists, contained lecithin, and there had been some doubt as to their nature. Valenciennes & Frémy had opposed the view that they were crystals, basing their view on Sennarmont's work, but Radlkofer and Hoppe-Seyler returned to the crystal theory. Hoppe-Seyler believed that vitellin contained no phosphorus, but that what appeared in the analyses was due to contamination with lecithin. This view was supported also by his assistant, Diakonov, who contributed to the *Med.Chem. Untersuchungen* one of the earliest investigations of phosphatide. But at the same time Miescher obtained from the yolk of the hen's egg a substance containing a great deal of phosphorus, and possessing certain of the properties of a protein. This he believed to be nuclein. "It is interesting ", he said, "in relation to the origin of nuclear substance, that the nutrient yolk contains ready-formed nuclein in significant quantity." At this time, then, the proteins of the yolk were believed to be ovoglobulin (for so Hoppe-Seyler called the vitellin of the earlier workers) and Miescher's nuclein. Miescher himself identified his nuclein as a constituent of the white yolk of the histologists, but he noted that the hen's egg seemed to have no xanthine in it.

Lehmann, Schwarzenbach and others, however, did not agree with this classification, and regarded vitellin as a mixture of albumen and casein. They did so not on the grounds of its containing phosphorus, but because they found that rennin would completely coagulate it from its pure solution. But this attitude did not prevail, and the word "nucleovitellin" became general, until Kossel in 1886 found that, if vitellin was really a nuclein, it differed from all other such substances by giving no trace of xanthine after acid hydrolysis. On the other hand, true nuclein, he found, was present by the tenth day of development. Hall and Burian & Schur, Bessau and von Fellenberg confirmed this absence of purines from the fresh egg. In more recent times, Sendju and Mendel & Leavenworth have found exceedingly small amounts of true nucleoprotein (2 and 1·6 mgm. per cent. respectively wet weight) in the hen's egg (by purine bases), and Plimmer & Scott, and Heubner & Reeb have done the same (by phosphorus analysis). Shortly after Kossel's work, Milroy found that vitellin gave a biuret test though no Millon, and materially differed in nitrogen and phosphorus content from any of the nucleoproteins, while, at the same time, Miescher admitted that he could not isolate any purine bases from his "nuclein" in the hen's egg. Levene & Alsberg next investigated the manner of breakdown of vitellin, finding the substance they named "paranuclein" after digestion with pepsin, and "avivitellic acid" after hydrolysing with ammonia. The elementary composition of these substances is given in Table 10 *a*, from which it could be seen that the increasing phosphorus content implied the presence of phosphorus as an important constituent of the original molecule. Six years later Levene & Alsberg ascertained the amino-acid distribution (see Table 11). They pointed out the significance of the high proline figure, in view of the task of haemoglobin synthesis which the young embryo has before it. Abderhalden & Hunter and Hugounenq undertook a like investigation in the same year, from which a striking similarity between the amino-acid distribution in vitellin and casein came to light, especially as regards the high proportion of leucine and glutamic acid. They drew attention to the similarity in physiological requirements as between the "erste Nahrung" of chick and mammal. The historical associations of this discovery have already been referred to (see p. 53). It was at this time that Neuberg, and Blumenthal & Mayer reported the existence of glucosamine in the vitellin molecule, two observations which stood together in isolation, until in 1929 Levene & Mori isolated from egg-yolk the same trisaccharide which they found to be present in ovoalbumen and ovomucoid and which has been referred to above.

It was not until the paper of Bayliss & Plimmer in 1906 that the



HEN'S EGG 1 MM. IN DIAMETER, NOT YET LIBERATED FROM THE OVARY

Stain, haemalum-eosin: magnification, 9 × A: prepared and microphotographed by Dr V. Marza. The stratification of the yolk into white and yellow is beginning.

nature of vitellin really became clear. They subjected casein and vitellin to the action of trypsin, and studied the time taken under varying conditions for the phosphorus to be split off into soluble form. Ovovitellin, they found, was much more slowly digested than casein, for after 36 days only half of its phosphorus had been made soluble, whereas after 2 or 3 days a large percentage of the casein phosphorus had gone into solution in inorganic form, and most of the rest was present in water-soluble organic combination. 1 per cent. soda, however, would bring all the phosphorus of casein into solution in 24 hours. Bayliss & Plimmer concluded that ovovitellin and casein were both phosphoproteins, as distinguished from nucleoprotein, where the phosphorus would be present in the prosthetic group and not in the protein itself. Plimmer & Scott later found that ovovitellin behaved in the same way to soda. This reaction served to distinguish between phosphoproteins and nucleoproteins, for all the latter, it was found, were stable to alkali and easily split by acids. From the phosphorus distribution in the unincubated hen's egg, Plimmer & Scott concluded that vitellin accounted for at least 30 per cent. of the phosphorus, and this led them on to their investigation of the changes which take place in the different phosphorus fractions during the development of the embryo.

The distribution of phosphorus-containing compounds in egg-yolk, as Plimmer & Scott found, makes a very different picture from that of any other tissue. Their summary is shown in the accompanying table (18). It would be extremely interesting to investigate the phosphorus distribution in the white yolk, which at present is altogether uncharted.

Table 18. *Phosphorus in per cent. of the total phosphorus.*

	Hen egg-yolk	Ox testis	Ox thymus	Ox pancreas
Lecithin P	61·4	33·7	14·3	11·5
Total water-soluble P ...	9·5	43·5	41·3	43·5
Water-soluble inorganic P...	None	27·9	21·1	23·0
Nucleoprotein P	1·6	22·8	44·4	41·9
Phosphoprotein P	27·5	None	None	3·1
Total protein P	29·1	22·8	44·4	45·0

The third of these fractions includes the phosphorus of all unstable water-soluble compounds.

Steudel, Ellinghaus & Gottschalk have recently found that vitellin behaves towards pepsin exactly like casein. The rate of increase of titratable COOH groups during the digestion far exceeds that of NH_2 groups, reaching a maximum about the fourth hour. The

Table 19.

	Per cent. of dry weight			
	Total N	P	Fe	N/P ratio
Plimmer's figures.				
Ovovitellin	15·29	1·0	—	15·3/1
Ovolivetin	15·12	0·1	—	151·0/1
Ovoalbumen	15·51	0·1	—	155·0/1
Casein	15·30	—	—	—
Levene & Alsberg's figures.				
Avivitellic acid ...	13·13	9·88	0·57	1·32/1
Swigel & Posternak's figures.				
Ovotyrine α_1	10·87	13·76	None	1·75/1
Ovotyrine β_1	11·33	12·55	None	2·00/1
Ovotyrine β_2	10·92	12·09	3·31	2·00/1
Ovotyrine γ_1	10·70	7·90	None	3·00/1

Swigel & Posternak's figures.

		Hydrolysis of ovotyrine b_1 (%)				
H_3PO_4	Pyruvic acid	NH_3	Arginine	Histidine	Lysine	*l*-serine
12·00	1·60	4·90	0·62	0·70	0·75	7·90

Table 20. *Nitrogen distribution.*

Plimmer's figures (1908).

	Per cent of dry weight				
	Total N	Amide N	Humin N	Diamino N	Mono-amino N
Ovoalbumen ...	15·51	1·34	0·29	3·30	10·58
Casein	15·30	1·52	0·22	3·30	10·36
Ovovitellin	15·29	0·84	0·25	3·84	10·26
Ovolivetin	15·12	0·75	0·32	3·29	10·76

linkages must break, therefore, between a carboxyl group and proline, tryptophane, histidine or arginine.

Weyl; v. Moraczevski; and Gross were the first to describe the properties of the egg-yolk proteins, but the standard account is that of Plimmer, who in 1908 identified two yolk-proteins, ovovitellin, and ovolivetin. Ovovitellin, according to his analyses, contained 1·0 per cent. of phosphorus, but ovolivetin only 0·1 per cent. He was usually able to isolate far more of the former than the latter, but in some experiments the yield seemed to be nearly equal. Livetin was soluble in water as well as 10 per cent. salt solution, but it cannot be ovoalbumen or any of the egg-white proteins, for it is not coagulated by ether. Plimmer suggested that possibly livetin was vitellin with the majority of the phosphorus-containing parts split off from it. Tables 19, 20 contain Plimmer's figures for these two proteins.

Table 21.

Tryptophane.

	May & Rose (1922), (%)	Folin & Looney (1922), (%)
Casein	1·5	1·54
Lactalbumen	2·4	—
Gliadin	1·05	1·14
Glutenin	1·8	1·68
Edestin	1·5	1·4
Gelatin	—	—
Ovovitellin	1·74	—
Ovoalbumen	1·11	1·23

Free tryptophane (% of whole egg)

	Ide (1921)	von Fürth & Lieben (1922)
Whole egg-contents ...	0·359	—
White	0·310	—
Yolk	0·437	—

Total tryptophane.

	(% of proteins)	(% of proteins)
Whole egg-contents ...	2·74	—
White	2·83	2·0
Yolk	2·51	2·1
		(% of egg)
Whole egg-contents ...	—	0·23

Ovovitellin has been the subject of recent investigations by Swigel & Posternak. They found that it broke up into three polypeptides which they call ovotyrine α_1, β_1 and γ_1. The properties of these are listed in Table 19. It was found that ovotyrine β contained all the iron in the original compound, and that it could be split up into ovotyrine β_1 and ovotyrine β_2 the second of which again contained all the iron. All these derivatives were laevorotatory, and showed considerable resemblance to the lactotyrines which the same workers had previously isolated from casein. They identified their ovotyrine β with the avivitellic acid of Levene & Alsberg, and they stated that an enzyme was present in the fresh yolk which would, on standing at 37° C. for 10 days, double the yield of preformed ovotyrine β.

Table 22. *Data concerning egg-fats.*

Material	Iodine no.	Saponification no.	Acid value	Ester value	Acetyl no.	Hehner no.	Refractive index	Sp. g.	% N	% C	Distribution of fatty acids						Investigator and date
											% butyric	% oleic	% palmitic	% stearic	% hydroxy stearic	% linolic	
Neutral fats																	
Hen (W. Leghorn) ...	64·84	182·5	5·98	177·5	—	78·41	—	—	—	—	—	3·01	—	—	—	—	Pennington (1910)
,, (Plym. Rock) ...	62·80	179·9	5·80	171·2	—	76·10	1·4626	—	—	—	—	2·92	—	—	—	—	,,
,, ...	72·0	190·0	1·20	—	—	95·16	—	—	—	—	—	81·6	9·6	0·6	6·4	—	Kitt (1897)
,, ...	81·4	186·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Paladino & Losso (1896)
,, ...	52·01	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Vignon & Meunier (1904)
Duck	37·36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Hen	64·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Reinsch & Bohn (1902)
Hen (on barley ration)	76·2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Henriques & Hansen (1903)
,, (on pea ration)	82·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on rice ration)	74·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on hemp ration)	120·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on linseed ration)	97·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on lipoid-free ration)	52·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	McCollum, Halpin & Drescher (1912)
,, (on ordinary ration)	64·2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Hen (Buff Orpington)	86·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Mottram (1913)
,, ...	70·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Eaves (1910)
Goose	63·3	199·2	4·7	194·5	—	87·1	1·4651	—	—	—	2·57	—	—	—	—	—	Hepburn & Katz (1927)
Duck	70·8	205·1	4·8	200·3	—	87·46	1·4674	—	—	—	2·66	—	—	—	—	—	,,

Hen	—	—	—	—	—	—	—	—	—	71·67	—	40·0	38·0	15·2	—	—	Liebermann (1888)
,, ...	—	—	—	—	—	—	—	—	—	76·91	—	—	—	—	—	—	Bohr & Hasselbalch (1903)
,, ...	75·0	187	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Griffith
Pigeon	70·8	173	—	—	—	—	—	—	—	—	—	—	—	—	—	—	McClure & Carr (1925)
Sea-urchin (*Echinometra lucunter*)	84·63	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Tennent, Smith & Gardiner (1928)
LECITHIN FATTY ACIDS																	
Hen	69·0	—	—		—	—	—	—	—	—	—	—	—	—	—	—	Rollett (1909)
,, ...	48·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Stern & Thierfelder (1907)
,, ...	82·31	198·95	—	—	3·82	—	—	—	—	—	—	—	—	—	—	—	Serono & Palozzi (1911)
Hen (on barley ration)	72·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Henriques & Hansen (1903)
,, (on pea ration)	70·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on rice ration)	66·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on hemp ration)	74·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on linseed ration)	78·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on lipoid-free ration)	34·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	McCollum, Halpin & Drescher (1912)
,, (on ordinary ration)	63·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,, (on hemp ration)	111·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Terroine & Belin (1927)
,, (on potato and corn ration)	98·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Hen	—	—	—	—	—	—	—	—	1·95	—	—	33·0	28·5	14·2	—	24·0	Cousin (1903)
Sea-urchin (*E. lucunter*)																	
(lecithin)	107·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Tennent, Smith & Gardiner (1928)
(cephalin)	46·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
SQUALENE																	
Various elasmobranchs	177·6	107·0	—	91·3	—	—	1·4769	0·8997	—	—	—	—	—	—	—	—	Tsujimoto (1920)

Hydrolysis of ovotyrine β revealed the presence of large amounts of *l*-serine, an amino-acid which had not previously been found in ovovitellin (see e.g. Plimmer & Rosedale's analyses). Some pyruvic acid and ammonia being given off as well, Swigel & Posternak calculated that, supposing these arose from breakdown of serine, there would have been sufficient serine present initially to combine with for all the phosphorus. They therefore suggested that the main phosphorus-containing unit of ovovitellin was serine-phosphoric acid.

Cohn, Hendry & Prentiss consider the minimal molecular weight of vitellin to be 192,000, i.e. much higher than ovoalbumen.

Kay & Marshall have also studied the yolk-proteins. They have prepared purer samples of vitellin and livetin than those of any previous worker, and have been able to free the former almost entirely from contamination with ovolecithin. Their vitellin is a true phosphoprotein containing 1·3 per cent. of phosphorus and hydrolysed by 1 per cent. soda, though not by the phosphatase of the kidney. Their livetin is a pseudo-globulin, containing only the slightest traces of phosphorus (less than 0·05 per cent.). The yolk of the fresh egg contains no albumen. Vitellin, hydrolysed with dilute ammonia, gives a vitellinic acid containing about 10 per cent. of phosphorus. Kay has estimated the cystine, tryptophane and tyrosine in vitellin and livetin (Table 11); in the latter protein they are distinctly high in amount, a fact of some importance in embryonic nutrition. The relative amounts of vitellin and livetin in yolk would appear to be of the order of 3·6 to 1 for the hen and 3·8 to 1 for the duck, calculating from their nitrogen content. Kay regards livetin as identical with Gross' protein. The yolk of a fresh egg would contain from 600 to 900 mgm.

1·9. The Fat and Carbohydrate of Avian Yolk

The fatty acids of the yolk have been much investigated since the time of Gobley and Kodweiss, but little has been added to our knowledge of them. Paladino found olein, palmitin and stearin to be present. Analytical details are in Table 22.

A large part of the study of phosphatides, under the generic name of lecithin, has been made on that obtained from the yolk of the egg; thus the work of Diaconov, who showed it contained no neurine, Strecker, who discovered the presence of choline, Bergell; Cousin; Laves & Grohmann; Laves; Wintgen & Keller; Erlandsen; Stern

& Thierfelder; Frankel & Bolaffio (whose egg-yolk neottin was only a mixture of sphingomyelin and cerebrosides), McLean; Serono & Palozzi; Eppler; Riedel; Wilson, and Trier, who prepared amino-ethylalcohol from it, all comes under this heading. In McLean's book will be found a review of it. Certain aspects of it, however, are important here; for instance, the question of the presence of very unsaturated acids in ovolecithin. McLean in 1909 found stearic and oleic acids in it, but Cousin was able to isolate linolenic and palmitic as well, and Riedel; Hatakeyama; and Levene & Rolf obtained linolic and arachidonic acids. In another paper Levene & Rolf showed that the lecithin, carefully freed from kephalin, contained only palmitic, stearic, and oleic acids: saturated and unsaturated molecules being present in equal proportions. Again, Stephenson in 1912 found an acid in the phosphatide fraction from egg-yolk, which had 20 carbon atoms and 6 or 8 unsaturated linkages. Although the proportion of unsaturated acids in egg-yolk is generally agreed to be small, yet it may be of importance for the young embryo if it passes through a period in the early developmental stages before it has the power of desaturating the ordinary fatty acids. Evidence which suggests this will be presented later (Section 11·1).

The nitrogenous radicle in ovolecithin is largely choline, but difficulty was at first experienced in obtaining a theoretical yield on hydrolysis; thus Moruzzi got only 77 per cent. in 1908 and McLean only 65 per cent. in 1909. This was accounted for, however, when it was found that amino-ethyl alcohol was also present. The two bases together make up all the nitrogen in the molecule. Erlandsen was the first to question the view that lecithin alone accounted for the phosphatide fraction, but he was not himself able to isolate anything else. Later workers (Levene & West and Stern & Thierfelder), however, found that kephalin is also present in yolk, and it would probably be in the kephalin molecule that the unsaturated fatty acids would be present. Analyses of kephalin from the yolks of fowls are given in Table 10 *b*. McLean in 1909 isolated from egg-yolk a third phosphatide which resembled cuorin, but it is very doubtful whether this was a true chemical individual. Sphingomyelin has also been found in egg-yolk by Levene (1916), and lignoceric as well as hydroxy-stearic acid was present in it.

All these substances exist in the yolk in close association with the proteins. Hoppe-Seyler it was who first observed that, after prolonged extraction of the yolk with ether, a considerable proportion of the phosphatides still remained behind, and could be extracted with alcohol. It was thought for a long time that the phosphatides and the vitellin were in chemical combination which was broken by the alcohol, but since the paper of Fischer & Hooker in 1916 the general opinion has been that this combination is only physical. Stern & Thierfelder isolated traces of the cerebrosides, phrenosin and kerasin, from egg-yolk in 1907.

The neutral fats and the lipoids of the yolk are variously affected by the nature of the fats in the food of the fowl. Henriques & Hansen, who were the first to investigate this subject, found that, if food containing very unsaturated acids was fed to the laying hens, the neutral fats in the eggs were affected, but not the fatty acid components of the lecithin. Their figures are shown in Table 22. When the food consisted of barley, pea or rice, the iodine number of the neutral fats in the egg varied round about 77, but hemp or linseed sent it up to about 110, although no matter what the food was the iodine number of the fatty acids in the phosphatide fraction remained constant at 75 or so. Henriques & Hansen also found that the iodine number of the fluid fatty acids of the neutral fat was normally 107·5, and that the fluid and solid fatty acids of the phosphatide fraction were 151·3 and 98·9 respectively. The former accounted for 64·3 per cent. of the lecithin fatty acids. The experiments of Henriques & Hansen have been repeated and confirmed by Belin and by Terroine & Belin. The last-named workers, together with McCollum, Halpin & Drescher, some years later reported that the lecithin fatty acids would vary, as well as the neutral fatty acids, with the diet of the hen. Their figures, which are given in Table 22, certainly show a variation in the iodine numbers of both fractions. All these workers recognised the presence of unsaturated acids in the yolk fat, and Henriques and Hansen's figures came between the theoretical values for oleic and linolic acids.

Work was continued along these lines by McClure & Carr. Using pigeons, they found that the fat content of the eggs could only be altered slightly by feeding rations high and low in fat.

	% fat in the eggs
Cocoanut fat	4·0
Beef tallow	6·75
Average of all fat diets	4·96
Average of all non-fat diets ...	4·81

Table 23. *Distribution of nitrogen in mgm. per cent.*

Wet weight data in ordinary figures; dry weight in italic figures.

Investigator and date	Species	EGG-WHITE								WHOLE EGG						YOLK						
		Total N	Non-coagulable protein N	Non-coagulable non-protein N	Coagulable protein N	Total NH_3 N	Total amino N	Albumose N	Free amino N	Total N	Non-coagulable protein N	Coagulable protein N	Free amino N	Basic N	Free ammonia N	Total N	Non-coagulable protein N	Non-coagulable non-protein N	Coagulable protein N	Total NH_3 N	Total amino N	Free amino N
Vladimirov & Schmidt (1926)	Hen	1,750	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sakuragi (1917)	Hen	—	—	—	—	—	—	—	—	2055	209	1846	—	—	—	—	—	—	—	—	—	—
Needham (1927)	Hen	—	—	8	—	—	—	—	—	2050	90	1960	—	—	—	—	—	—	—	—	—	—
Aggazzotti (1919)	Hen	1,730	—	—	—	146	1300	—	16	—	—	—	—	—	—	2600	—	—	—	206	1740	58
Cook (1908)	Hen	1,760	299	—	1,460	—	—	—	36	—	—	—	—	—	—	2620	136	—	2479	—	—	92
,,	Hen	*12,695*	*2152*	—	*10,537*	—	—	—	*260*	—	—	—	—	—	—	*4954*	*270*	—	*4684*	—	—	*174*
Pennington (1910)	Hen *	1,680	153	—	1,540	—	—	40	13	—	—	—	—	—	—	—	—	—	—	—	—	—
,,	Hen *	*14,250*	—	—	*13,560*	—	—	*478*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
,,	Hen †	1,700	171	—	1,540	—	—	75	5	—	—	—	—	—	—	—	—	—	—	—	—	—
,,	Hen †	*14,260*	—	—	*13,320*	—	—	*418*	*43*	—	—	—	—	—	—	—	—	—	—	—	—	—
Kojo (1911)	Hen	1,750	—	—	—	—	—	—	—	—	—	—	—	—	—	2490	—	—	—	—	—	—
Bywaters (1913)	Hen	2,130	310	—	1,820	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Idzumi (1924)	Hen	—	—	—	—	—	—	—	—	2021	36	1914	20	—	—	—	—	—	—	—	—	—
Tomita (1921)	Hen	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	18
Levene (1902)	Hen	—	—	—	—	—	—	—	—	2000	428	1320	—	252	—	—	—	—	—	—	—	—
Houghton & Weber (1913)	Hen	—	—	—	—	—	—	—	—	—	—	—	—	—	12	—	—	—	—	—	—	—
Hendrickson & Swan (1918) ‡	Hen	—	—	—	—	—	—	—	—	—	—	—	—	—	1·3	—	—	—	—	—	—	—
Pucher (1928) §	Hen	—	—	7·34	—	—	—	—	3·28	—	—	—	—	—	—	—	—	66·7	—	—	—	49
Leveque & von Tschermak (1913)	Hen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	*5030*	—	—	—	—	—	—

* White Leghorn. † Plymouth Rock.

‡ Hendrickson & Swan also reported 3·0 mgm. % wet weight of free ammonia nitrogen in the yolk and 0·4 mgm. % wet weight in the white.

§ Pucher also reported 6·49 mgm. % urea N and 0·20 mgm. % uric acid N (wet weight) in the yolk and corresponding values of 1·92 mgm. and 0·20 mgm. in the white.

Again, during the fat feeding, the saponification value of the egg-fat was 176 (166–190) and during the rest of the time 173. The iodine value was in the former case 70·5 and in the latter 70·8.

Table 24. *Lipoid in egg-yolk.*

		Lecithin	
		% Wet weight	% Dry weight
Laves	Hen	8·90	16·80
Manasse	,,	9·41	17·80
Parke	,,	10·70	18·40
Barro	,,	9·16	—
Glikin	,,	10·16	17·36
,,	Pigeon	13·11	28·76

Glikin's figures.

	Dry substance %	Total fatty acids % dry substance	P_2O_5 in % of fatty acids	Lecithin in % of fatty acids	Lecithin in % dry weight
Pigeon (yolk)	45·75	65·07	3·16	35·73	23·37
	45·63	62·89	3·88	38·42	24·16
Turtledove (yolk) ...	17·95	44·06	4·10	46·65	20·55
Starling (whole egg) ...	—	28·26	5·67	64·44	18·21
Hen (yolk)	54·31	—	—	—	13·71

	Lecithin in % dry weight at birth or hatching
Thrush	8·18
Cat	5·06
Rabbit	4·91
Guinea-pig	3·79

Some suggestive investigations on the biological significance of ovolecithin were made by Glikin in 1908, whose figures are shown in Table 24. Choosing the pigeon as a typically nidicolous bird, and the hen as a typically nidifugous one, he was able to show, using a variety of extraction methods, that the yolk of the former was considerably richer in lecithin than the latter, the former containing about 29 per cent. dry weight, and the latter about 17. The further but rather fragmentary observations which he made on the starling and the turtledove confirmed this relationship. It is interesting that Tso informs us that certain small Chinese breeds of hen produce very small eggs (scarcely 40 gm.) and that these contain a much higher percentage of lipoids than ordinary eggs though an equivalent percentage of protein. He concluded that lecithin, one of the most essential yolk-constituents, was specially concentrated in nidicolous yolks and was associated with the property of early hatching or birth. Thus he compared the thrush (nidicolous) with the guinea-pig, which is born almost ready to eat green food and hardly passes through a lactation stage; in the body of the former 8 gm. per cent. lecithin dry weight was found, in the latter only 4. The new-born cat and rabbit occupied intermediate positions. It is interesting to note that Glikin's figures bear out those of Tarchanov on the question of water-content of yolks from the two types of birds.

Tornani affirmed in 1909 that a difference in lecithin-content was observable between fertilised and unfertilised eggs. But as he gave no figures in support of his contention, it has not been treated with much respect by subsequent workers.

The carbohydrate of the yolk has been the subject of only a very few researches compared with that of the white. The figures which have been obtained are shown in Table 16, and it will be seen that in no case has the total carbohydrate been estimated, and only in one case the glycogen. After Claude Bernard's isolation of glycogen from the yolk, a persistent belief grew up that considerable amounts of this substance were present there; this was apparently based on the description by Dareste in 1879 of "amyloid bodies" in the yolk which gave microchemically a strong blue colour with iodine. Dastre immediately pointed out that the occurrence of starch there was highly improbable, and that if any glycogen was there it should give a wine-red colour; he himself, however, could find neither. But he did not succeed in suppressing the rumour, for Virchow, and later Schenk, supported Dareste, though nothing has been heard of this yolk-constituent since 1897, and Sakuragi's analysis revealed the presence of only 2·2 mgm. per cent. of glycogen. Bierry, Hazard & Ranc asserted in 1912 that they could obtain a great increase of carbohydrate after hydrolysing the yolk with hydrofluoric acid under pressure, but this would not imply, as they seemed to think, that glycogen was present, for all kinds of other compounds such as proteins (Gross' protein for instance) might yield glucosamine under such treatment. They identified glucosamine in the hydrolysate. On the other hand, Diamare, who hydrolysed with acetic acid, could only obtain faint traces of combined glucose in the yolk. He dialysed both white and yolk, and in both cases was able to estimate the free sugar, but in the case of the yolk very little combined glucose seemed to be present. Further studies on this subject should be undertaken, for the methods of Diamare and Bierry alike were of questionable reliability. Diamare, however, went rather further into the matter than other investigators, and, thinking that the yolk glucose might only be present there owing to an inflow from the white, examined the ovarian eggs, in which he found glucose in much the same proportion as in the yolks of laid eggs. He does not state whether the ovarian eggs were yellow or white, and, as he frequently gives his results in the form of grams of glucose without mentioning the weight of the fresh material, it is impossible to calculate the percentage (see also Tillmans & Philippi).

We have already seen that cholesterol was identified in the yolk of the hen's egg by very early workers such as Gobley. In 1908 Menozzi and in 1915 Berg & Angerhausen showed that egg cholesterol was identical with that from milk and bile. It is certainly present in the unincubated yolk both free and in esterified form with fatty acids. Serono and Palozzi investigated a substance from egg-yolk in 1911 which they called "lutein" but which turned out to be nothing but a mixture of cholesterol esters. Other investigators have estimated the amount of free and combined cholesterol in the unincubated egg, and their figures are given in Table 25.

Table 25. *Cholesterol-content of hen's egg.*

	Mgm. per whole egg			
Investigator and date	Cholesterol (free)	Cholesterol esters	Total	Free in % of total
Parke (1866)	—	—	248·3	—
Mendel & Leavenworth (1908)	378	—	—	—
Mueller (1915)	215·9	24·2	240·1	89·92
Ellis & Gardner (1909)... ...	489	—	600·6	80·6
Thannhauser & Schaber (1923)	173	54·2	227	76·8
Cappenberg (1909)	—	—	180	—
Dam (1929)	296	—	—	88·87
Schönheimer (1929)	337	—	—	—

Many other substances have been found to be present in the egg at the beginning of development, e.g. choline, alcohol, creatine, creatinine, inositogen, lactic acid, plasmalogen (Stepp, Feulgen & Voit, 1927), etc. These will be mentioned as occasion arises during the succeeding sections of the book. Allantoin is not present (Ackroyd).

The yolk of the hen's egg also contains vitamines, pigments, and a variety of enzymes, but these will be dealt with under their respective sections. As Langworthy has shown, it may also contain very

Table 26. *Distribution of phosphorus in the avian egg.*

Wet weight data in ordinary figures; dry weight in italic figures.

	Mgm. % wet weight								Mgm. per egg								% of total P						
Species	Total P	Ether-soluble P	Alcohol-soluble P (after ether extraction)	Total alcohol-soluble P	Total water-soluble P	Organic water-soluble P	Inorganic water-soluble P	Vitellin P	Total P	Ether-soluble P	Alcohol-soluble P (after ether extraction)	Total alcohol-soluble P	Total water-soluble P	Organic water-soluble P	Inorganic water-soluble P	Vitellin P	Phosphatide P	Phospho-protein P	Total water-soluble P	Organic water-soluble P	Inorganic water-soluble P	Nuclein P	Investigator and date
Whole egg																							
Hen	—		←335·4→		—	—		237·6	—		←167·7→			—		118·8	—	—	—	—	—	—	Maxwell (1893)
,,	357	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Mansfeld (1901)
,,	443	—	—	—	—	—	—	—	214	—	—	—	—	—	—	—	—	—	—	—	—	—	Juckenack (1899)
,,	—	367	—	—	—	—	—	—	—	168	—	—	—	—	—	—	—	—	—	—	—	—	Lührig (1904)
,,	*1845*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Gobley (1846)
,,	215	—	—	—	—	—	—	—	107·5	—	—	—	—	—	—	—	64·8	27·1	—	6·2	Trace	1·9	Plimmer & Scott (1909)
,,	*1455*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Löbisch
,,	226·8	—	—	—	—	—	—	—	113·4	72	—	—	9·7	5·1	4·5	31·7	63·2	28·0	8·8	4·8	4·0	None	Masai & Fukutomi (1923)
,,	550	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Lebbin (1904)
,,	*1580*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,,	—	—	—	—	—	—	Trace	—	—	—	—	—	—	—	Trace	—	—	—	—	—	—	—	Forbes, Beegle & Mensching (1914)
,,	—	—	—	—	—	—	—	—	197·0*	—	—	—	—	—	—	—	—	—	—	—	—	—	Russell & McDonald (1929)
,,	—	757	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Vignon & Meunier (1904)
Duck	—	742	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
,,	—	—	—	—	—	—	—	—	—	199	—	—	—	—	Trace	152	56·8	43	—	—	—	—	Fingerling (1912)
Hen	494	—	—	—	—	—	17·4	—	—	—	—	—	—	—	—	—	—	—	—	—	3·41	—	Chapin & Powicke (1915)
,,	*1915*	—	—	—	—	—	*67·2*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,,	480	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Delezenne & Fourneau (1918)
Peacock	478	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
Yolk																							
Hen	1220	—	—	770	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Carpiaux (1903)
,,	1440	306	617	923	—	—	—	—	660	140	283	423	—	—	—	—	85·3	—	—	—	—	—	Juckenack & Posternak (1904)
,,	1279	478	345	—	—	—	—	456	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Lührig (1904)
Duck	1255	643	218	—	—	—	—	394	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Hen	1440	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Lebbin (1904)
,,	*2720*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
,,	1350	—	—	590	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	van Meurs (1923)
,,	1279	478	345	—	—	—	—	456	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Juckenack (1899)
,,	*2950*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Leveque & Ponscarme (1913)
,,	*2620*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Leveque & von Tschermak (1913)
White																							
Hen	15	3	None	None	—	3	9		—	—	—	—	—	—	—	—	—	—	—	—	—	—	Heubner & Rees (1910)
,,	*110*	*20*	,,	,,	—	*20*	*70*		—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
,,	31	—	—	—	—	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	van Meurs (1923)
,,	155	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Kaas (1906)
,,	31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Juckenack (1899)

N.B.—The organic water-soluble phosphorus includes pyrophosphate P and the inorganic water-soluble phosphorus includes any guanidine-phosphoric acids which rapidly hydrolyse (e.g. phosphagen).

* plus 16·1 mgm. in the shell.

various substances derived from the diet of the hen, and these, if they are odorous or possess taste may very easily betray their presence (e.g. the Swedish "Schäreneier" described by Hansson). Table 23 gives the figures which are available for the nitrogen and Table 26 for the phosphorus distribution. These summaries bear out on a detailed basis what has already been said.

1·10. The Ash of the Avian Egg

The ash of the yolk and the white of the hen's egg has been investigated by several workers, and a study of it reveals certain interesting features. If Table 27 be examined, it will be seen that, in the yolk as well as the white, potassium has almost invariably been found to be present in greater amount than sodium. This is one of the characteristics of the egg-cell, as will be seen later when the eggs of other animals are considered. The yolk is also marked by the very high percentage of phosphorus, most of which is, in accordance with other evidence, in organic combination. The calcium is also mainly in the yolk, as is the iron, but not the magnesium. If now the amounts of metallic and acidic ion be calculated out in millimols and milliequivalents per cent. wet weight, it is found that in both yolk and white there is an uneven balance, but while in the former case there is much more anion than cation (anion/cation ratio above unity), in the latter case the exact reverse holds, and the anion/cation ratio is somewhat below unity, about 0·55. In the white, therefore, some of the potassium and sodium must be combined with the proteins, as ovoalbumenates, etc.* However, the excess of cation over anion in the white is not so considerable as the excess of anion over cation in the yolk, and, bearing in mind also the much higher percentage of solid in the yolk than in the white, it would be expected that the anion/cation ratio of the whole egg would be greater than unity, and would approach that of the yolk. The facts show that this is, indeed, the case, for the average anion/cation ratio calculated from the results of all observers for the whole egg is 2·3, as against 2·8 for the yolk alone and 0·54 for the white alone. This was first noted by Carpiaux. Forbes, Beegle & Mensching expressed it simply thus:

	Cubic centimetres normal solution per 100 gm. dry weight egg
Total acid	120·28
Total base	39·42
Excess acid over base	80·86

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* In both white and yolk, of course, the *inorganic* ash is basic.

Table 27. *Ash of the avian egg.*

Egg	Ash % wet weight	% of total ash								Mgm./100 gm. wet weight								Millimols								Milliequivalents								Total cation		Total anion		Anion/cation ratio	Investigator and date
		K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	Milli-mols	Milli-equiv.	Milli-mols	Milli-equiv.		
Whole																																							
Hen	1·543	6·5	4·05	1·17	8·5	—	—	79·8	—	101	62·5	18	131	—	—	1231	—	2·59	2·72	0·75	3·28	—	—	10·3	—	2·59	2·72	1·50	6·56	—	—	30·9	—	9·34	*13·37*	10·3	*30·9*	2·31	Poleck (1850)
,, ...	1·437	9·05	0·76	1·32	9·7	—	—	79·1	—	130	11	19	140	—	—	1137	—	3·33	0·48	0·79	3·5	—	—	11·9	—	3·33	0·48	1·58	7·0	—	—	35·7	—	8·1	*12·39*	11·9	*35·7*	2·88	Rose (1850)
,, ...	1·725	10·0	1·22	1·16	8·1	—	—	64·2	15·2	175	21	20	140	—	—	1107	262	4·5	0·91	0·83	3·5	—	—	11·65	7·38	4·5	0·91	1·66	7·0	—	—	34·95	7·38	9·79	*14·11*	19·03	*42·33*	3·00	Bialascewicz (1926)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	80	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Plimmer & Lowndes (1927)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	139	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Vaughan & Bills (1878)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Delezenne & Fourneau (1918)
Peacock ..	—	—	—	—	—	—	—	—	—	—	—	—	69	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
Duck .	—	—	—	—	—	—	—	—	—	—	—	—	65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
Hen ...	1·20	14·4	16·97	0·68	7·78	—	0·32	50·3	8·98	173	204	8	93	—	3·8	604	108	4·43	8·8	0·34	2·3	—	0·04	6·36	3·02	4·43	8·8	0·68	4·6	—	0·08	19·08	3·02	15·87	*18·51*	9·42	*22·18*	1·20	Carpiaux (1908)
,, ...	0·91		—	1·29	7·97	—	—	41·86	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Buckner & Martin (1920)
,, ...	—	5·96	11·3	1·7	—	—	22·0	24·8	18·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
																																					Average ...	2·3	
Hen Normandy	0·125	17·5	27·3	1·0	9·3	—	2·1	36·8	6·1	219	314	12·5	116	—	26	460	76	5·61	14·8	0·52	2·91	—	0·27	4·8	2·1	5·61	14·8	1·04	5·82	—	0·54	14·4	2·1	23·84	*27·27*	7·17	*17·04*	0·625	Champion & Pellet (1876) (not very reliable)
Bourbonnais	0·130	15·4	23·8	Trace	15·0	—	3·5	35·7	6·9	200	309	—	195	—	45	460	89	5·13	13·5	—	4·87	—	0·47	4·8	2·5	5·13	13·5	—	9·74		0·94	14·4	2·5	23·50	*28·37*	7·77	*17·84*	0·629	,,
White																																							
Hen	0·590	23·55	20·7	0·96	1·24	—	2·63	6·46	25·25	139	122	5·7	7·3	—	15·5	38	149	3·56	5·3	0·24	0·18	—	0·16	0·4	4·2	3·56	5·3	0·48	0·36	—	0·32	1·2	4·2	9·28	*9·70*	4·76	*5·72*	0·58	Poleck (1850)
,, ...	0·720	27·66	12·1	2·7	2·9	0·38	1·7	3·16	15·5	199	87	19	21	2·7	12·2	22·7	112	5·11	3·8	0·81	0·52	0·05	0·13	0·24	3·14	5·11	3·8	1·62	1·04	0·15	0·26	0·72	3·14	10·29	*11·72*	3·51	*4·12*	0·35	Rose (1850)
,, ...	0·634	—		24·8	25·5	6·83	—	43·5	—	—	—	15·5	16	4·2	—	27·6	—	—	—	0·65	0·40	0·075	—	0·3	—	—	—	1·30	0·80	0·225	—	0·9	—	—	—	—	—	—	Voit (1877)
,, ...	0·600	22·1	20·63	1·41	1·64	—	1·9	5·22	26·1	132·6	123·8	8·5	9·8	—	11·4	31·3	157	3·4	5·4	0·35	0·24	—	0·12	0·3	4·4	3·4	5·4	0·7	0·48	—	0·24	0·9	4·4	9·39	*9·98*	4·82	*5·54*	0·55	Iljin (1917)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Prout (1822)
,, ...	—	—	—	—	4·25*	—	—	—	—	129·2	52·5	—	—	—	—	—	160·5	—	—	—	—	—	—	—	—	3·73	2·61	—	—	—	—	—	5·15	—	—	—	—	—	Straub & Hoogerduyn (1929)
																																					Average ...	0·54	
Yolk																																							
Hen	1·523	4·68	3·79	1·24	8·72	—	—	85·5	4·25	71·2	57·7	19	133	—	—	1305	64·7	1·8	2·5	0·79	3·3	—	—	13·7	1·8	1·8	2·5	1·58	6·6	—	—	40·1	1·8	8·39	*12·48*	15·5	*41·9*	3·4	Poleck (1850)
,, ...	1·345	10·9	1·08	2·2	13·62	—		60·16	3·6	146·5	14·5	29·6	183	—	—	810	48·4	3·7	0·63	1·24	4·5	—	—	8·5	1·4	3·7	0·63	2·48	9·0	—	—	25·5	1·4	10·07	*15·81*	9·9	*26·9*	1·7	Rose (1850)
,, ...	1·525	—	—	1·86	12·70	0·87	—	84·6	—	—	—	28	193	13	—	1290	—	—	—	1·2	4·8	0·23	—	13·6	—	—	—	2·4	9·6	0·69	—	40·8	—	6·23	*12·69*	13·6	*40·8*	3·2	Voit (1877)
,, ...	2·20	—	—	—	—	—	—	—	—	←— 770 —→			210	—	—	1220	—	—	—	—	5·2	—	—	12·8	—	—	—	—	10·4	—	—	38·4	—	—	—	—	—	—	Carpiaux (1903)
,, ...	1·10	7·01	4·34	1·23	9·28	—	—	87·4	—	77·1	47·7	13·5	102	—	—	961	—	1·9	2·1	0·56	2·5	—	—	10·1	—	1·9	2·1	1·12	5·0	—	—	30·3	—	7·06	*10·12*	10·1	*30·3*	2·9	Iljin (1917)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	68	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Prout (1822)
,, ...	—	—	—	—	—	—	—	—	—	103·9	101·7	—	—	—	—	—	139·8	—	—	—	—	—	—	—	—	5·81	9·3	—	—	—	—	—	7·89	—	—	—	—	—	Straub & Hoogerduyn (1929)
																																					Average ...	2·8	

*Kreis & Studinger.

This is probably the most interesting consideration that emerges from Table 27, but it may also be noted that the ash-content of the white is just about half that of the yolk, a relation which would practically be reduced to equality if the phosphorus in the yolk was not taken into account.

The presence of certain chemical elements of lesser biological importance has been announced from time to time in a group of papers which have some interest, although it is difficult to see, as yet, what their importance is for the development of the embryo. Fluorine has been estimated by Tammann and by Nicklès, copper by Dhéré, boron by Bertrand & Agulhon, manganese by Bertrand & Medigreceanu, iodine by Bonnanni and by von Fellenberg, lead by Bishop.

These elements appear to be normal constituents of the egg. The iron-content can be artificially increased by feeding iron-rich rations to the hen, and iodine can also be introduced into the egg in this way, as has been done by Bonnanni, Kreis and others, but Hofmann found that though iron and iodine would enter the egg thus, it was impossible to get copper to do so. In just the same way Ricci found it difficult if not impossible to get As or Hg into the hen's egg by feeding subtoxic doses to the hen. The normal copper-content of the hen's egg cannot be varied like its iron-content. The importance of iron in the formation of haemoglobin is obvious, and the little that is known about this process will be discussed in the section on pigments in the embryo. Wassermann made a histochemical examination of the egg-yolk and vitelline membrane for iron, and found a relationship between the embryonic blood-islands and the iron of the yolk.

Some of the other data in Table 28 call for comment. Tammann's 1·13 mgm. per cent. fluorine in the fresh yolk works out at a quantity of 0·2 mgm. per egg, and, as Zaleski found 0·23 per cent. fluorine in the bones of the chick at hatching, 0·08 mgm. fluorine would be required in the egg at the beginning, or less than half of what is actually there. Zaleski's figure, however, is old, and may be too low. It would appear, on the whole, as if the greater part of the fluorine, iodine, copper, zinc, lead, aluminium, silicon and manganese is localised in the yolk, and the greater part of the boron and arsenic in the white. In view of the importance which we now attribute to these less common elements as catalysts in living tissues, this distribution may be found to have considerable significance.

Table 28. *Rarer elements in eggs.*

Figures in ordinary type indicate absolute amounts per egg or part of an egg in mgm.: in italics indicate mgm. % wet or dry weight.

Element	Species	Whole egg	Shell	White	Yolk	Investigator and date
Fluorine	Hen	—	Practically none	Present but in insufficient amount for analysis	*1·13* (wet)	Tammann (1888)
	,,	—	—	Present	—	Nicklés (1856)
Iodine	Hen	0·013 *0·31* (wet)	—	—	—	v. Fellenberg (1923)
	,,	0·02	—	Traces only	—	Bonnanni (1912)
	,,	0·017	—	—	—	Bleyer (1926)
	,,	0·005–0·020	—	—	—	Rowett Research Institute (1929)
	,,	0·009–0·013	—	—	—	Hercus & Roberts (1927)
	,,	0·07	—	—	0·18	Cruickshank (1929)
	Trout	0·01 *10·3* (wet)	—	—	—	v. Fellenberg (1923)
Boron	Hen	—	—	*0·1* (dry)	*0·0016* (dry)	Bertrand & Agulhon (1913)
	,,	—	—	*0·014* (wet)	*0·0008* (wet)	,, ,,
	Goose	—	—	*0·1* (dry)	—	,, ,,
	Turkey	—	—	*0·1* (dry)	—	,, ,,
			Present in shell and shell-membranes of all eggs studied			
Copper	Octopus (*Sepia officinalis*)	0·005–0·008	—	—	—	Dhéré (1904)
	Hen	—	—	*0·56* (dry)	*0·76* (dry)	Elvehjem, Kemmerer, Hart, & Halpin (1929)
	,,	—	—	Trace	—	Wolff (1929)
	,,	*0·82* (dry)	—	—	*0·8* (dry)	Lindow, Elvehjem & Peterson (1929)
	,,	*0·23* (wet)	—	—	*0·4* (wet)	,,
	,,	—	—	—	*2·0* (dry)	Fleurent & Levi (1920)
	,,	—	—	—	*0·6* (dry)	McHargue (1925)
	Human foetal brain	Present	—	—	—	Bodanski (1921)

MANGANESE	Hen	Present, more in the yolk than in the white	—	—	—	Pechard (1898 & 1900)
	"	—	—	Less than *0·002* wet weight, prob. absent	*0·063* (wet)	Bertrand & Medigreceanu (1913)
	Duck	—	—	—	*0·054* (wet)	Bertrand & Medigreceanu (1913)
	Herring	—	—	—	*0·083* (wet)	" "
ZINC	Herring	—	—	—	*2·8* (wet)	Bertrand & Vladesco (1921)
	"	—	—	—	*12·4* (dry)	" "
	Hen	*1·4* (wet)	None	None	*4·9* (wet)	Bertrand & Vladesco (1922)
	"	—	"	"	*9·9* (dry)	" "
	"	*1·58* (wet)	"	Trace	*5·01*	Birckner (1919)
	"	—	—	None	*0·4* (wet)	Wolff (1923)
	"	0·5	—	—	—	Rost (1920)
	Human foetal brain	Present	—	—	—	Bodanski (1921)
SILICON*	Hen	—	—	0·49	0·55	Poleck (1850)
	"	—	—	0·28	0·62	Rose (1850)
	"	—	—	1·14	—	Iljin (1917)
ARSENIC	Hen	0·005 (from 50 to 75 % of this in the yolk)	—	—	—	Gautier (1900); Bertrand (1900)
	"	*0·013* (wet) *0·050* (dry)	—	*0·007* (wet) *0·054* (dry)	*0·023* (wet) *0·048* (dry)	v. Fellenberg (1930)
LEAD	Hen	—	*0·1–1·6* (wet)	*0·12–0·48* (wet)	*0·2–1·0* (wet)	Bishop (1928)† and Bishop & Cooksey (1929)
URANIUM	Hen	—	Trace	—	—	" "
LITHIUM	Hen	—	"	—	—	" "
ALUMINIUM	Hen	*0·017* (wet)	—	—	—	Underhill, Peterman, Gross & Krause (1929)
	"	—	—	None	0·02—0·06	Wolff, Voorstram & Schoenmaker (1923)
	"	—	—	*0·03* (wet)	*0·47* (wet)	Smith (1928)

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* All figures for silicon refer to mgm. SiO_3 % of total ash.

† During the development, all the lead of the egg-contents enters the embryo, and at 11·5 days there is 0·05–0·5 mgm. % wet weight in it. Bishop considers that this is present in combination with lecithin and partly as lead oleate.

The figures which have been obtained by those investigators who have examined the iron-content of eggs are seen in Table 29. All found a great deal more iron in the yolk than in the white, as might have been expected from the earlier micro-chemical researches of Tirmann and Kobert. This kind of work had been originated by Schmiechovski, and was continued later by Wassermann in the interesting paper already referred to. Schmiechovski found iron histochemically throughout the yolk, but considered that, in the white or milky yolk, it was confined to the megaspheres.

Table 29. *Iron in hen's eggs.*

Italic figures indicate dry weight data.

Fe_2O_3 gm. % wet weight

Without iron-rich diet				With iron-rich diet				
Egg-white	Yolk	Egg-white plus yolk	Shell	Egg-white	Yolk	Egg-white plus yolk	Shell	Investigator and date
·0024	·0088	·0047	·0272	·0040	·0095	·0059	·0272	Loges & Pingel (1901)
—	—	·0046	—	—	—	·0040	—	Kreis (1900)
·0057	·026	·0165	—	—	—	—	—	Lebbin (1900)
·03	*·05*	*·03*	—	—	—	—	—	,,
·00112	·00995	·00425	—	—	—	—	—	Hartung (Mar. 1902)
·00087	·01106	·00451	—	—	—	—	—	,, (May 1902)
—	—	—	—	·00208	·01621	·00729	—	,, (June 1902)
Trace	·0108	—	—	—	—	—	—	Bunge (1892)
—	·0121	·0018	—	—	·0175	·0032	—	Hofmann (1901)
—	—	·0057	—	—	—	—	—	Boussingault (1850)
—	*·063*	*·095*	—	—	—	—	—	Leveque & von Tschermak (1913)
None	*·0143*	—	—	None	*·0143*	—	—	Elvehjem, Kemmerer, Hart, & Halpin (1929)

Wassermann, using both the ammonium sulphide and the Berlin blue methods, decided that it was present in both kinds of yolk, but that it was not confined to those special elements in the white part. In fact, it was very much more abundant in the white than in the yellow part. This finding has never been corroborated by chemical analysis, but, if it is, it will have considerable importance, in view of the time at which haemoglobin is most vigorously manufactured by the embryo. For a further discussion of these questions see the section on pigments.

1·11. General Characteristics of Non-Avian Eggs

With this we may conclude the discussion of what is known about the typically terrestrial egg, that of the bird. Now aquatic species far outnumber the terrestrial ones; as Spenser put it:

> O! What an endlesse Worke have I in hand
> To count the sea's abundant progeny,
> Whose fruitfull seede farre passeth those on land,
> And also those which wonne in th' azure sky:
> For much more eath to tell the starres on hy,
> Albe they endlesse seeme in estimation,
> Than to recount the sea's posterity,
> So fertile be the flouds in generation,
> So huge their numbers, and so numberlesse their nation.

It might therefore be supposed that a much greater space would have to be devoted to their eggs than what has already been taken up, but this is not the case, for the bird's egg has been so convenient a material for research that the knowledge we have of it outweighs that of the eggs of all other animals put together. Indeed, the data about the eggs of other groups are very fragmentary, so that much caution has to be used in making comparisons, and general relationships are much more difficult to enunciate. Van Beneden's classical memoir may be recommended as an account of the morphology of the eggs which are to be mentioned, and it is hardly necessary to refer to Balfour's book on comparative embryology.

If Table 30 is examined, and compared with Table 2, it will at once be seen that the percentage composition of eggs of other classes of animals differs markedly and in very definite ways from the egg of the hen. The case of reptiles may first be taken, as being less remote than others. The reptilian egg seems to be distinctly drier than that of the bird, by about 20 per cent., and much more variable in its fat/protein ratio. For, while in all birds' eggs that have been investigated, the amount of protein, whether related to dry or to wet weight, is about the same as that of fat, the reptilian egg shows big variations from this rule. In the eggs of the tortoise and lizard, for example, there is twice as much protein as fat, while in those of the grass-snake, studied by Galimard, there is three times as much fat as protein. This fact will be mentioned again later (see p. 313). Considerably more is known however about those of amphibia, which have also been found to contain a great deal more protein than fat. Thus, instead of the 40 per cent. protein and the 40 per cent. fat which make up the dry substance of the hen's egg, Fauré-

Table 30. *General constitution of eggs (excluding those of birds).*

		% wet weight									% dry weight								
Material	Weight of the egg gm.	Water	Protein	Fat	Non-nitrogenous extractives	Ash	Lipoids	Cholesterol	Total unsaponifiable fraction	Phosphorus	Protein	Fat	Nitrogen	Non-nitrogenous extractives	Ash	Lipoids	Cholesterol	Phosphorus	Investigator and date
REPTILES																			
Grass-snake (*Coluber natrix*)	—	—	—	8·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Hilger (1873)
Tortoise	—	71·15	23·69	10·45	—	1·64	—	—	—	—	—	—	—	—	—	—	—	—	Greshov, Sack & van Eck (1903)
Lizard (*Varanus salvator*) ...	—	72·77	14·31	9·87	—	2·20	—	—	—	—	—	—	—	—	—	—	—	—	" " "
Grass-snake (*Tropidonotus natrix*)	—	58·94	6·50	21·81	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Galimard (1904)
" " ...	—	48·13	6·90	41·80	—	6·70	—	—	—	—	—	—	—	—	—	—	—	—	Sommer & Wetzel (1904)
AMPHIBIA																			
Frog (*Rana esculenta*) ...	—	57·37	—	11·92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Kolb (1901)
Frog (*Rana temporaria*) ...	0·0034	57·60	26·51	10·14	3·31	—	—	—	—	—	60·0	14·82	—	—	—	5·98	—	—	Fauré-Fremiet & Dragoiu (1923)
Frog (*Rana fusca*)	—	59·3	27·9	8·57	—	—	—	0·62	1·68	—	—	—	11·0	—	—	—	—	—	Terroine & Barthélemy (1921)
Frog (*Rana esculenta*) ...	—	—	—	11·00	—	—	—	0·71	1·83	—	—	—	—	—	—	—	—	—	" "
" " ...	—	—	—	10·30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Terroine & Barthélemy (1923)
Frog (*Rana temporaria*) ...	—	—	—	11·90	—	—	—	1·62	—	2·05	—	—	—	—	—	—	—	—	Parnas & Krasinska (1921)
" " ...	—	57·6	26·51	—	11·0	—	3·31	—	—	—	—	3·9	—	—	—	20·0	—	—	Fauré-Fremiet & Vivier du Streel (1921)
" " ...	0·0022	—	—	—	—	—	—	—	—	—	53·2	12·65	8·51	—	—	2·15	—	—	Barthélemy & Bonnet (1923)
" " ...	—	55·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	von Bezold (1857)
" " ... (with jellies)	—	78·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Bialascewicz (1908)
" " ...	—	58·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Williams (1900)
" " ... (with jellies)	—	68·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Needham (1927)
" " ...	—	55·7	13·45	10·41	13·8	1·78	—	—	—	—	—	—	—	—	—	—	—	—	Baudrimont & Martin de St Ange (1846)
" " ...	—	52·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Wetzel (1908)
Salamander (*Cryptobranchus alleghaniensis*)	—	—	—	—	—	—	—	—	—	—	69·0	30·57	—	—	—	—	—	—	Gortner (1914)
" " ...	0·128	54·7	—	—	—	—	—	—	—	—	—	17·5	—	—	—	—	—	—	McClendon (1915)

Fishes

Sturgeon	—	56·97	27·87	12·85	1·62	2·31	—	—	—	—	64·77	29·86	9·36	—	—	—	—	—	Kostytschev (1883)
,, ...	—	53·84	25·18	13·12	—	7·86	—	—	—	—	69·11	28·42	8·73	—	—	—	—	—	Lidov (1880)
,, ...	—	62·82	23·19	9·24	3·01	1·74	—	—	—	—	62·37	24·84	10·0	—	4·68	—	—	—	Buttenberg (1900)
,, ...	—	61·84	22·37	10·61	3·49	1·69	—	—	—	0·6	58·62	27·80	9·38	—	4·43	—	—	—	,,
,, ...	—	52·2	27·00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Linnert (1909)
Gar (*Lepidosteus platystomus*)	—	53·9	26·2	18·3	1·54	0·42	—	—	—	—	—	—	—	—	—	—	—	—	Nelson & Greene (1921)
Shad (*Clupea sapidissima*) ...	—	71·2	23·4	3·8	—	1·6	—	—	—	—	—	—	—	—	—	—	—	—	Atwater (1882)
Swedish cod (*Brosmius vulgaris*)	—	66·03	29·92	2·26	—	2·16	—	—	—	—	88·07	6·65	14·08	—	6·36	—	—	—	Solberg (1908)
Cod (*Gadus merlangus*) ...	—	72·15	23·42	1·54	1·08	1·81	—	—	—	—	83·98	5·51	13·44	—	6·44	—	—	—	König & Grossfeld (1912)
Cod	—	70·36	—	1·81	2·36	2·05	—	—	—	—	80·91	6·22	13·52	7·97	6·93	—	—	—	Rimini (1903)
,, ...	0·0097	94·67(?)	—	—	—	—	—	—	—	—	68·09	—	10·90	—	—	10·09	—	2·65	Levene (1899)
Carp	—	72·17	19·78	—	6·85	0·91	—	—	—	—	71·03	24·05	11·36	—	—	—	—	—	Popov (1897)
,, ...	—	66·15	27·68	2·48	2·29	1·40	—	—	—	—	81·80	7·32	13·09	—	4·13	—	—	—	König & Grossfeld (1913)
,, ...	—	64·08	14·23	2·57	0·44	0·82	3·24	—	—	—	—	—	—	—	—	—	—	—	Gobley (1850)
Carp (*Cyprinus carpio*) ...	—	66·30	25·70	6·20	None	2·00	—	—	—	—	—	6·08	—	—	—	12·33	1·34	—	Fauré-Fremiet & Garrault (1922)
Plaice (*Pleuronectes platessa*)	0·0033	92·90	6·34	0·085	—	—	—	—	—	—	88·9	1·19	—	—	—	—	—	—	Dakin & Dakin (1925)
Herring	—	69·22	26·31	3·19	—	1·38	—	—	—	—	84·31	4·02	13·49	—	3·83	—	—	—	König & Splittgerber (1908)
,, ...	—	60·0	—	—	—	—	4·88	—	—	—	—	—	—	—	—	13·88	—	—	Rewald (1929)
,, ...	—	65·0	29·49	3·62	—	—	—	—	—	—	81·47	10·33	—	—	—	6·53	—	—	Hugounenq (1904)
,, ...	0·004	71·71	13·44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Steudel & Osato (1923)
,, ...	—	—	—	—	—	—	—	—	—	—	—	1·25	12·06	—	—	—	—	—	Yoshimura (1913)
Pike	—	63·53	28·12	1·40	4·89	2·06	—	—	—	—	77·10	3·84	12·33	—	5·65	—	—	0·60	König & Grossfeld (1913)
,, ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11·67	—	0·44	Vageler (1909)
,, ...	—	68·50	—	—	—	—	—	—	—	—	50·0	—	—	—	—	—	—	—	McCrudden (1921)
Trout (*Savelinus fontinalis*)	—	63·85	27·81	3·71	3·00	1·63	—	—	—	—	76·93	10·26	12·31	—	4·51	—	—	—	König & Grossfeld (1913)
Perch (*Perca flavescens*) ...	—	56·28	14·00	2·97	—	0·92	—	—	—	—	64·8	14·9	10·3	—	4·65	—	—	—	Pearse (1919)
Trout (*Savelinus fontinalis*)	0·062	72·6	—	—	—	—	—	—	—	—	—	16·17	—	—	—	—	—	—	McClendon (1915)
Trout	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10·83	—	0·41	Vageler (1909)
Trout (*Savelinus fontinalis*)	—	67·17	—	—	—	1·25	—	—	—	—	—	—	—	—	3·8	—	—	—	Pearse (1919)
,, ,,	0·02	—	11·62	3·31	—	—	—	—	—	—	—	—	11·5	—	—	—	—	—	Gortner (1913)
Trout	—	66·12	25·45	7·25	—	—	—	—	—	—	—	12·0	—	—	—	—	—	—	Tangl & Farkas (1904)
Trout (*Salmo fario*) ...	—	67·10	23·83	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Kronfeld & Scheminzki (1926)
,, ,, ...	—	58·50	29·31	9·16	0·34	1·25	—	—	—	—	—	10·0	—	—	—	8·2	1·37	—	Fauré-Fremiet & Garrault (1922)
Bullhead	—	—	—	—	9·23	4·82	—	—	—	—	—	—	—	—	—	—	—	—	Rimini (1903)
Tunnyfish	—	—	32·25	17·97	1·39	3·67	—	—	—	—	—	—	—	5·78	—	—	—	—	,,
King-salmon (*Oncorhyncus tschtschawei*)	—	57·68	26·56	11·70	1·43	0·68	1·90	—	—	—	—	—	—	—	—	—	—	—	Greene (1921)
Salmon	—	—	—	—	—	—	—	—	—	—	87·8	4·5	—	—	—	7·5	2·2	—	Kensington (1884)
,, ...	—	63·0	25·2	10·50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Paton & Dunlop (1898)
Dogfish (*Scyllium canicula*)	5·2	48·6	—	—	—	—	—	—	—	—	75·0	30·0	12·0	—	5·5	—	—	2·7	Wetzel (1907)
Dogfish (*Squalus acanthias*)	102	52·68	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Zdarek (1904)
Dogfish (*Scyllium canicula*)																			
Whole egg	—	53·15	23·58	22·34	—	0·93	—	—	—	0·87	—	—	—	—	—	—	—	—	Ford & Thorpe (1920)
Egg-white	—	79·54	5·62	14·42	—	0·42	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
Yolk	—	46·40	28·28	24·25	—	1·07	—	—	—	—	—	—	—	—	—	—	—	—	,, ,,
Dogfish (*Squalus acanthias*) (egg-white or jelly)	—	88·75	4·27	5·06	—	1·87	—	—	—	—	—	—	—	—	—	—	—	—	Perugia (1879)

Table 30. *General constitution of eggs* (*excluding those of birds*) [cont.].

Material	Weight of the egg gm.	% wet weight									% dry weight								Investigator and date
		Water	Protein	Fat	Non-nitrogenous extractives	Ash	Lipoids	Cholesterol	Total unsaponifiable fraction	Phosphorus	Protein	Fat	Nitrogen	Non-nitrogenous extractives	Ash	Lipoids	Cholesterol	Phosphorus	
FISHES (*cont.*)																			
Dogfish (*Mustelus laevis*) ... (egg-white or jelly)	—	92·10	5·14	3·02	—	0·63	—	—	—	—	—	—	—	—	—	—	—	—	Perugia (1879)
Dogfish (*Galeus canis*) (egg-white or jelly)	—	93·80	1·69	3·65	—	0·84	—	—	—	—	—	—	—	—	—	—	—	—	,,
Barbel	—	73·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	McCrudden (1921)
Pelagic eggs:																			
Plaice	0·0032	91·66	5·15	—	—	—	—	—	—	0·14	—	—	—	—	—	—	—	—	Milroy (1898)
Whiting	0·0009	96·41	2·58	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Cod	0·0015	91·14	5·95	—	—	—	—	—	—	0·16	—	—	—	—	—	—	—	—	,,
Haddock	0·0026	95·86	3·42	—	—	—	—	—	—	0·11	—	—	—	—	—	—	—	—	,,
Brill	0·0006	90·00	5·53	—	—	—	—	—	—	0·13	—	—	—	—	—	—	—	—	,,
Flounder	0·0006	87·20	8·33	—	—	—	—	—	—	0·17	—	—	—	—	—	—	—	—	,,
Ling	0·0006	85·07	7·61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Demersal eggs:																			
Herring	—	70·04	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Milroy (1898)
Herring (*Cottus scorpio*) ...	—	74·46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Catfish	—	71·30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Lumpsucker	—	70·46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Goosefish (*Lophius piscatorius*)	—	68·70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Salmon	—	56·70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Trout	—	60·90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
Eel	—	57·70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,
ECHINODERMS																			
Sea-urchin (*Paracentr. lividus*)	—	80·50	—	—	—	—	—	—	—	—	—	—	8·60	—	—	—	—	—	Russo (1926)
,, ,,	—	—	—	—	—	—	—	—	—	—	—	14·80	—	—	—	2·15	—	—	Meyerhof & von Schönborn (1911)

,, ,,	—	77·30	—	4·81	—	0·34	—	—	—	—	66·88	21·20	10·70	>5·7	1·5	—	—	—	Ephrussi & Rapkine (1928)
,, ,,	—	77·90	10·15	4·32	—	2·19	—	—	—	0·27	45·0	19·10	7·20	—	9·4	—	—	2·1	Wetzel (1907)
Sea-urchin (*Echinus esculenta*)	—	82·80	—	8·68	—	—	3·62	—	—	—	—	—	—	—	—	—	—	—	Moore, Whitley & Adams (1913)
Sea-urchin (*Arbacia punctulata*)	—	81·90	5·85	2·25	2·20	0·04	—	—	—	0·66	—	—	—	—	—	—	—	—	McClendon (1909)
Sea-urchin (*Echinometra lucunter*)	—	—	—	9·1	—	—	—	0·69	—	—	—	—	—	—	—	—	—	—	Tennent, Smith & Gardiner (1928)
Starfish (*Asterias glacialis*) ...	—	81·50	11·82	4·00	—	1·90	—	—	—	—	—	—	—	—	—	—	—	—	Dalcq (1923)
CRUSTACEA																			
Spider-crab (*Maia squinado*)	—	56·40	—	—	—	—	—	—	—	—	65·0	24·10	10·40	—	4·12	—	—	3·0	Wetzel (1907)
ARACHNIDS																			
King-crab (*Limulus polyphemus*)	—	49·36	19·25	6·20	—	1·97	—	—	—	—	—	—	—	—	—	—	—	—	Greshov, Sack & van Eck (1903)
INSECTS AND ARACHNIDS																			
Silkworm moth (*Bombyx mori*)	0·006	64·5	19·30	8·08	5·8	—	1·04	0·4	—	—	—	—	—	—	—	—	—	—	Tichomirov (1882)
,, ,,	—	63·97	26·48	8·15	—	1·23	—	—	—	—	—	31·9	9·1	—	4·5	—	—	—	Farkas (1903)
,, ,,	—	—	—	—	—	—	—	—	—	—	—	7·21	—	>3·08	—	—	—	—	Vaney & Conte (1911)
,, ,, ...	—	66·24	22·0	—	—	—	—	—	—	—	—	—	10·88	—	—	—	—	—	Russo (1922)
Acarid parasite (*Margaropus australis*)	0·0009	59·0	—	—	—	—	—	—	—	84·0	—	7·0	—	>7·0	2·0	—	—	—	Fauré-Fremiet & Garrault (1928)
Fly (*Calliphora vomitoria*) ...	0·001	76·0	—	4·90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Hofmann (1872) and Weinland (1908)
Lackey-moth (*Malacosoma americana*)	—	48·5	—	—	—	—	—	—	—	71·90	—	4·45*	11·5	—	—	—	—	—	Rudolfs (1926)
MOLLUSCS																			
Octopus (*Sepia officinalis*) ...	0·065	52·70	—	—	—	—	—	—	—	69·0	—	12·30	10·70	—	2·2	—	—	2·3	Wetzel (1907)
,, ,,	0·077	52·50	—	—	—	2·2	—	—	—	—	—	—	—	—	—	—	—	—	Ranzi (1929)
Limpet (*Patella vulgata*) ...	—	67·0	16·83	13·53	—	0·95	—	—	—	—	—	29·0	—	—	—	11·0	—	—	Fauré-Fremiet & Garrault (1923)
Brazilian shellfish (*Voluta brasiliense*)	—	74·52	—	—	—	16·0 (!)	—	—	—	—	—	—	—	—	—	—	—	—	Roffo & Correa (1927)
ANNELIDS																			
Worm (*Sabellaria alveolata*)	—	70·0	19·08	6·80	—	1·53	—	—	—	—	—	—	8·50	—	—	2·50	3·90	—	Fauré-Fremiet (1921)
NEMATODES																			
Roundworm (*Ascaris megalocephala*)	—	—	11·10	—	—	—	—	—	—	—	—	—	1·78	—	—	—	—	—	Kozmina (1928)
,, ,,	—	69·50	—	—	—	—	—	—	—	—	—	—	—	21·05	1·5	20·0	—	—	Fauré-Fremiet (1913

* This figure would be 9·0 if the egg-cases were excluded.

Fremiet & Dragoiu found the dry material of the egg of the frog to consist of 60 per cent. protein and only 14 per cent. fat. And this has been the experience of all those who have analysed amphibian eggs.

Next to the hen's egg, the eggs of fishes have probably received the most attention. The recorded analytical figures for fish eggs are often deceptive, for many analyses have been made of salted fish roes and egg-preparations such as caviar, but the greatest care has been taken not to include in Table 30 results which might have been vitiated in that way. The question is complicated by the fact that analyses of the purified constituent egg-substances prepared from preserved material may well be admitted into consideration, for, except in certain cases, they would probably not undergo much change during the process of preservation. As in the case of reptiles and amphibia, the fish egg is characterised by its predominance of proteins as the food of the growing embryo. It should be remembered that, in all these comparisons, the yolk of the hen's egg is a more proper standard of reference than the whole egg-contents, in which case the differences become even more remarkable. This generalisation appears at all points; thus, the brook-trout, a fresh-water fish, has 30 per cent. of protein and only 9 per cent. of fat, and the herring has 26 per cent. as against 3 per cent. In Table 31 the protein/fat ratios are collected together, and the difference emerges there with great clearness. Though it is obvious that fishes vary considerably among themselves as to the fat-content of their eggs, yet all of them have more protein than fat. The only fishes in Table 31 which come near to being exceptions to this rule are the sturgeon and the dogfish, both of which have an unusually high amount of fat in their eggs (a fact which accounts for the superiority of Russian caviar over other varieties, for the former is made chiefly from the eggs of sturgeon). Besides this general difference between the egg of the bird and that of the fish, there are many others, but they concern the chemistry of the individual components rather than the rough constitution of the egg as a whole, and will, therefore, be dealt with later on. If Table 31 be further studied, it will be seen that, as far as can be known at present from the few analyses of crustacean and cephalopod eggs, the superior proportion of protein over fat holds good there also. Curiously enough, the only analysis we have for a gastropod egg gives a picture more resembling the egg of the hen, comparatively equal amounts of fat and protein being present.

The position of affairs may perhaps be summarised by saying that it is only the birds which have been successful in producing an egg really well stocked with fat, though the reptiles clearly show an approximation to this achievement. Does this mean that the storage of fat in the egg is particularly associated with terrestrial embryos? The facts and arguments to be brought forward in later chapters (see Sections 7·7, 9·15 and 11·8) make this hypothesis a very likely one, but, as Table 31 shows, the silkworm (the only representative of terrestrial arthropods) does not seem to have succeeded in storing fat in its egg to any great extent. It might, of course, be argued that this was one of the factors which prevented the insects attaining any considerable size and rivaling reptiles and mammals for the possession of the land. The mammals gave up the heavy fat storage in the egg when they invented viviparity and the fully developed placenta. In

Table 31. *Protein/fat ratio in various eggs.*

	VERTEBRATA	
AMNIOTA		
Aves	Hen (yolk)	0·450
	,, (whole egg)	1·035
Reptilia	Lizard	1·450
	Grass-snake	0·298
	Tortoise	2·271
ANAMNIA		
Amphibia	Frog	2·610
Pisces	Cod	12·550
	Sturgeon	1·937
	Herring	8·333
	Carp	9·101
	Trout	3·518
	Dogfish	1·050
	Salmon	2·400
	INVERTEBRATA	
ECHINODERMATA		
Echinoidea	Sea-urchin	2·370
MOLLUSCA		
Cephalopoda	Octopus	5·750
Gastropoda	Limpet	1·245
ARTHROPODA		
Crustacea	Crab	2·709
Arachnida	King-crab	3·166
Insecta	Silkworm	2·139
ANNELIDA		
Polychaeta	*Sabellaria*	3·162

this connection the monotreme egg would be a chemical study of great interest, and it is characteristic of the exasperating fragmentation of this field of work that all we know about the monotreme egg is that its membrane seems to have the properties of a keratin. The suggestion that the metabolism of the fowl, operating on a continuously high level of energy turnover, would naturally tend to fill up the eggs with fat, and is associated with the well-known higher temperature of the avian body (Wetmore) may not be without value, but any special emphasis on fat metabolism in adult birds is precluded by the statements in Schulz's review.

It is very significant that as animals became more complicated and more adaptable to varied surroundings, higher, in fact, in the taxonomic scale, they loaded their eggs to a greater extent with yolk. Since the extra material was usually fatty acids, this process appears strikingly in Table 31. The effects of the yolk have long been familiar to embryologists, and have been best described, perhaps, in a passage by Milnes-Marshall. "The immediate effect of a large amount of yolk", he said, "is to retard mechanically the processes of development, but the ultimate result is to shorten them. This paradox is readily explained. A small egg, such as that of *Amphioxus*, starts its development rapidly, and in about eighteen hours gives rise to a free-swimming larva, capable of independent existence, with a digestive cavity and a nervous system already formed; while a large egg such as that of the hen, hampered by the great mass of yolk by which it is distended, has, in the same time, made very little progress. From this time onwards, however, other considerations begin to tell. *Amphioxus* has been able to make this rapid start owing to its relative freedom from yolk, but now this freedom becomes a retarding influence, for the larva, containing within itself but a very scanty supply of nourishment, must devote much of its energies to hunting for and to digesting, its food, and hence its further development will proceed more slowly. The chick embryo on the other hand has an abundant supply of food in the egg itself and has no occasion, therefore, to spend its time searching for it, but can devote its whole energies to the further stages of its development. Hence, except in the earliest stages, the chick develops more rapidly than *Amphioxus* and attains its adult form in a much shorter time. The tendency of abundant yolk to lead to shortening or omission of the ancestral history, is well known. The embryo of forms well provided with yolk takes short cuts in its development, and jumps from branch to branch of its genealogical tree instead of climbing steadily upwards. Thus the little West Indian frog, *Hylodes*, produces eggs which contain a larger amount of yolk than those of the ordinary English frog. The young *Hylodes* is consequently enabled to pass through the tadpole stage before hatching, and to attain the form of the frog before leaving the egg; the tadpole stage is, in fact, only imperfectly recapitulated, the formation of gills, for instance, being entirely omitted."

The more yolk, then, the longer the embryo can remain an embryo before having to face the external world, and the more preparations it can make for that event. It is probable that this question is intimately bound up with the penetration of fresh-water surroundings by the originally marine forms. "It has long been noticed", said Milnes-Marshall, following the classical exposition of Sollas, "that marine animals lay small eggs whereas their fresh-water allies lay eggs of much larger size. The eggs of the salmon or trout are much larger than those of the cod or the herring, and the crayfish, though only a quarter the length of the lobster, lays eggs of actually larger size. The larger size of the eggs of the fresh-water forms appears to be dependent on the nature of the environment to which they are exposed. Considering the geological instability of the land as compared with the ocean, there can be no doubt that the fresh-water fauna is, speaking generally, derived from the marine fauna, and the great problem with regard to fresh-water life is to explain why it is that so many groups of animals which flourish abundantly in the sea should have failed to establish themselves in fresh water. Sponges and Coelenterates abound in the sea, but their fresh-water representatives are extremely few in number; Echinoderms are exclusively marine; there are no fresh-water Cephalopods, no Ascidians, and of the smaller groups of Worms, Molluscs, and Crustacea, there are many that do not occur in fresh water. Direct experiment has shown that in many cases this distribution is not due to the inability of the adult animals to live in fresh water, and the real explanation appears to be that the early larval stages are unable to establish themselves under such conditions. To establish itself in fresh water permanently an animal must either be fixed, or else be strong enough to withstand and make headway against the currents of the streams or rivers it inhabits, for otherwise it will in the long run be swept out to sea, and this condition applies to larval forms equally with adults. The majority of marine invertebrates leave the egg as minute ciliated larvae, which are quite incapable of holding their own in currents of any strength. Hence it is only forms which have got rid of the free-swimming ciliated larval stage, and which leave the egg as organisms of considerable size and strength, that can establish themselves as fresh-water animals. This is effected most readily by the acquisition of yolk—hence the large size of the eggs of fresh-water animals—and is often supplemented by special devices."

Here is an explanation for the well-known paucity of eggs in fresh-water plankton. In certain cases it is possible to induce an embryo to skip the larval stage which it should normally pass through. Thus Child could abolish the free-swimming larval stage in the ascidian *Corella willmeriana*, simply by removing the eggs from the parental atrial chamber (*p*H 7·4) to normal sea-water (*p*H 8·4).

Giard had also noticed the discrepancy in egg-size between closely related marine and fresh-water forms, and had classed it among those cases where like adults have unlike larvae ("Poecilogony"). The classical instance is perhaps that of the shrimp *Palaemonetes varians*, one variety of which (*microgenitor*) lives in the sea near Wimereux and has eggs 0·5 mm. diam. (321 per female) and another of which (*macrogenitor*) lives in fresh water at Naples and has eggs 1·5 mm. diam. (25 per female). Giard has reviewed this subject in a very interesting paper. "Dans un groupe déterminé", he said (*Œuvres diverses*, p. 18), "la condensation embryogénique va en croissant des types marins aux types d'eau douce ou terrestres."

The correlated proposition, namely, that the fresh-water forms generally lay fewer eggs than the marine ones, is illustrated by the following instances collected by Carpenter:

	No. of eggs laid per female per annum			
	Marine form		*Fresh-water form*	
Lamellibranchs ...	*Ostrea edulis*	1,800,000	*Uniopictorum*	220,000
			Anodonta cygnea	18,000
Gastropods ...	*Buccinum undatum*	12,000	Average of many snails	100
			Average of many limpets	6
			Ovoviviparous pond-snails	15
Fishes	Haddock	9,000,000	Brook-trout	750
Crustacea ...	Lobster	5,000	Crayfish	200

Another reason for the poverty of fresh-water fauna was suggested by von Martens who pointed out that the fresh-water climate, with its periods of desiccation and freezing, was much more severe than that of the sea. But even these two causes together cannot fully account for the phenomenon, for there are many cases of individual species which they will not cover; thus the Cephalopods, which hatch out as minute but very active copies of their parents, i.e. which pass their larval stage within the egg, and which should therefore be immune from the disadvantage described by Sollas, never penetrated into fresh water.

A third reason must be added to those of Sollas and of von Martens. As will be shown in Sections 12 and 13 the marine invertebrate embryo depends largely on the salts of the sea water for its supply of ash, and therefore could not be expected to develop in a medium very poor in inorganic matter. Colonisation of the fresh water could not occur, then, until animals had begun to provide in each egg sufficient ash to make one finished embryo. There seem to be few data concerning the capacity of marine invertebrate eggs to develop in fresh water, although the adult animals have been found often enough to accustom themselves to a fresh-water environment (see the instances given in Semper). Many studies of the effect of hypotonic solutions on marine embryos can, however, be called to mind, and in all the cases the results are teratogenic.

The fate of the Cephalopods, it is interesting to note, is explained by this third factor, for Ranzi has demonstrated the intake of the salts in the sea water by the octopus egg.

As for the general statement that animals can afford their young a better chance of survival by providing them with larger amounts of yolk and therefore a longer incubation-period, there is a striking parallel here with the seeds of leguminous plants which are packed with nourishment. In the *Origin of Species* (6th ed. p. 56), Darwin wrote, "From the strong growth of young plants produced from such seeds as peas and beans when sown in the midst of long grass, it may be suspected that the chief use of the nutriment in the seed is to favour the growth of the seedlings, whilst struggling with other plants growing vigorously all round".

It is interesting that the birds show an adaptation exactly similar to the poecilogony of the invertebrates and fishes. Tree-nesting birds are usually nidicolous, but the defenceless state of the newly-hatched squab has brought it about that ground-nesting birds are usually nidifugous.

As Table 30 shows, the composition of the eggs of all animals other than those of the frog, the silkworm, and certain fishes, is still, to use a phrase of William Harvey's, "hid in obscurity and deep night". It is as yet much too early to try to draw any conclusions from the very fragmentary figures which are all that we have at our disposal, and we may well admit that one of the most urgent needs of chemical embryology is a much wider extension of our knowledge of the static chemistry of the egg. This is a quite indispensable preliminary to the investigation of the metabolism of the embryo in the lesser known forms. The attempt has already once been made to link up in some way the chemistry of the egg with what is known of the type of embryonic development which takes place in it. Wetzel in 1907 analysed the eggs of a sea-urchin, a crab, a cephalopod, and an elasmobranch fish. He pointed out that the eggs he studied were examples of varying richness in yolk, of total and partial, equal and unequal, superficial and discoidal cleavage, as well as chemical systems. Taking the egg of *Strongylocentrotus lividus* as his first case, he regarded it as typical of a class of alecithic eggs, of a total and equal cleavage type, and he drew attention to the fact that it was rich in water and in salts, but poor in fatty substances, in nitrogen, and in phosphorus. Similarly, in the case of the mollusca, where there is no very definite type of development, the egg of *Sepia* could not stand as representative of any wider class than the cephalopods, but, as far as it went, it showed that the cephalopod egg was rich in nitrogen, poor in fat and inorganic substances, with a moderate phosphorus and water-content. The decapod crustacea, to which *Maia squinado* belongs, have a purely superficial type of cleavage, with no cell-multiplication in that part of the egg which holds the yolk. Accordingly, the egg possessed a moderate fat and water-content, a moderate ash, and much protein and phosphorus.

The mammalian ovum is still as unknown chemically as it was when Wetzel was writing, and it may be found to have a constitution not unlike the alecithic echinoderm eggs. For the eggs of birds (and of reptiles, which only differ from them in having very little egg-white) Wetzel found a low protein and water-content, a high proportion of fat and ash, and a large amount of calcium and phosphorus. Here cleavage would only take place at one isolated point on the surface of the mass of food-material. In the amphibia, the richness of yolk, while much more significant than in lower classes, does not reach the level of birds and reptiles, and this is duly reflected in the chemical composition by the moderate water-content, the high proportion of protein which is yet only double that of the fat. The case of the dogfish is again different, for there the egg is rich in yolk and the cleavage is meroblastic; thus the water is rather low, the fat rather high, the nitrogen very high, and the ash and phosphorus moderate.

But these conclusions of Wetzel's, interesting though they are, cannot really be assessed until a great deal more comparative work has been done. They must rather be taken to represent the kind of correlation we may hope for in the future. However, one of Wetzel's generalisations may be accepted, if with some reserve. He pointed out that the fat-content of eggs showed great variations, rising from 12 per cent. of the dry weight of the *Sepia* egg to 66 per cent. of the dry weight of the (yolk of the) hen's egg. Again, the nitrogen gave very variable results, rising from 5·3 per cent. of the dry weight in the (yolk of the) hen's egg to 6·9 per cent. in the egg of the grass-snake, 12 per cent. in the egg of the dogfish, and even in the case of the cod 14 per cent. On the other hand, the phosphorus-content varied only between the (outside) limits of 2·1 per cent. for the sea-urchin egg and 3·6 per cent. for that of the grass-snake. Wetzel, therefore, suggested that a distinction might be made, at any rate, roughly, between those constituents of the egg which may serve as sources of energy for the growing embryo, and those which in no circumstances do so. Protein, fat, and carbohydrate would come in the former class; phosphorus (for nucleoprotein) and cholesterol, for example, would come in the latter class. The former would show great variations among eggs of different species, the latter would not. He thus supposed that one might be able to deduce, as it were, the constitution of any given egg, if one knew what substances, and in what proportions, were used by the embryo as combustible material during its development, as well as the constitution of the newly born or hatched organism.

From this standpoint Wetzel distinguished four types of substances in the unincubated egg: (1) material for the embryo to burn during the course of its development, (2) constituents of the finished protoplasm of the embryo, (3) constituents of the finished embryo, but not for incorporation into the protoplasm itself, but into the paraplasm (in Le Breton's terminology), (4) the protoplasm of the original egg-cell. No aspect of chemical embryology needs attention more urgently than this, and the correlation of chemical constitution with developmental type should offer a most attractive field for research.

But it is not only correlations of this type that lie hidden under the enigmatic character of analytical figures. The water-content of the eggs may have a powerful effect on the sex-ratio, for King found in 1912 that reducing the water-content of fertilised frog's eggs considerably lowered the proportion of males, while increasing it by means of treatment with dilute acid considerably raised the proportion. A discussion of these facts in relation to genetics as a whole will be found in the review of Huxley. It is probable that the effect which delayed fertilisation has upon the sex-ratio is to be explained by difference in water-content of the eggs. Hertwig was the first to observe this delayed fertilisation phenomenon in some work which he published in 1905, and since then it has many times been observed not only for amphibia but also for trout (Kuschakevitsch; Huxley; Mršic). Riddle has suggested that the mammalian egg may be subject to such influences as it passes from ovary to uterus. He quotes van der Stricht's histological work on the bat's egg during this process, and points out that the swelling of the yolk-granules would indicate an absorption of water. The exact degree of hydration of the mammalian egg might thus conceivably have an effect on the mammalian sex-ratio.

Table 30 has several more important points which have not, so far, been touched upon. It is interesting to follow in the figures of Milroy the difference between the fish eggs which float at the surface of the water during their development (pelagic ova), and those which sink, or rather float, at lower and denser levels (demersal ova)—the former have a water-content of about 90 per cent., the latter of about 70 per cent. A knowledge of the chemical composition of fish eggs throws a great deal of light upon their distribution in the sea, and so indirectly upon ecological problems. Their fat-content, for example, has been treated from this point of view by Polimanti, whose work will be discussed in the section on the general metabolism of the embryo; and the investigations of the specific gravity of fish eggs, which are discussed in Section 5, have also an important bearing upon these problems. Another point worth notice is the approximately constant percentage of cholesterol in different eggs, nearly always about 500 mgm. per cent. of the wet weight, a proportion which, roughly speaking, holds for the egg of the hen as well.

It would be as well to emphasise the fact that no principle of selection has been used in the preparation of Table 30, on the ground that results such as those of Roffo & Correa on a Brazilian gastropod, and McCrudden on fresh-water fishes, which seem obviously wrong, may not be so at all. The estimation methods and analytical processes which are by general consent judged most satisfactory at the present time cannot be considered in any way final, and to have excluded certain results on account of the technique employed in obtaining them would not have been justifiable. Table 30 does not, therefore, absolve investigators from the duty of looking up the original papers in such cases as touch them most closely, and forming an independent judgment, according to the best opinion of the time, on the stress which can be laid upon them. It is needless to say that I leave out of account all doubtful figures in the generalisations made here.

1·12. Egg-shells and Egg-membranes

Very little is known about the relative proportions of yolk, white, and shell, in the eggs of the lower animals, or rather, in most cases, egg-contents and shell or surrounding membrane. Table 32 gives a few figures. The discrepancy between the results of Ford & Thorpe, on the one hand, and Wetzel, on the other, is very strange, especially as they both used *Scyllium canicula* eggs, but it is probably due to insufficiency of the statistical element. Ford & Thorpe's proportions are more likely to be accurate.

Much work, however, has been done on the membranes and hard coverings which invest the unincubated eggs of different kinds of animals. For instance, the gelatinous substance which surrounds the undeveloped amphibian egg was examined chemically by Brande in 1810, who noticed that it absorbed water and was not precipitated by tannin or by strong acids. Later work has shown that it consists almost entirely of mucoprotein and water. Wetzel's figures for its weight are shown in Table 32. Giacosa isolated mucin in a pure state from it in 1882, and the figures which he obtained for its percentage composition are shown in Table 33. He was able to show the presence of a reducing sugar on hydrolysis, but he could isolate nothing else from the jelly, and therefore concluded that it was pure mucin. The presence of glucosamine in the mucoprotein was afterwards confirmed by Hammarsten, by Schulz & Ditthorn and by Wolfenden, who confirmed Giacosa's finding that

Table 32.

	In % of total egg-weight			
Species	Egg-membranes	White	Yolk	Investigator and date
Herring	2·4	—	—	König & Grossfeld (1913)
Carp ...	3·7	—	—	,, ,,
Cod ...	4·4	—	—	,, ,,
Pike ...	4·1	—		,, ,,
Dogfish	5·4	19·3	75·3	Ford & Thorpe (1920)
,,	26·9	36·5	36·5	Wetzel (1907)
Silkworm	8·87 (wet)	—	—	Tichomirov (1882)
,,	25·97 (dry)	—	—	,,
Trout ...	13·57–20·29	—	—	Kronfeld & Scheminzki (1926)
Octopus	86·0	—	14·0	Ranzi (1930)

Tomita's figures.

Marine turtle (*Thalassochelys cortica*)

	Weight in gm.	%
Shell	2·0	5·8
White	13·5	39·2
Yolk	18·9	55·0
Total	34·4	

Wetzel's figures.

Frog (*Rana temporaria*)

	Weight in mg.	%
Ovarial egg (no jelly)...	1·897	—
Egg with unswollen jelly	4·674	—
Jelly alone	2·777	—
Swollen jelly	8·97	—
Water content of ovarial egg ...	—	52·5
,, ,, jelly	—	78·65
,, ,, egg and jelly ...	—	67·48
Empty dry jelly	0·62	—
Dry egg	0·90	—
Dry egg + dry jelly	1·52	—
Thus of dry weight egg	—	59·28
,, ,, jelly	—	40·72

Melvin's figures.

Shell-weights of insects

	% of total weight of eggs
Squash-bug (*Anasa tristis*)	29·2
Luna moth (*Tropoeoa luna*)	23·3
Cecropia moth (*Samia cecropia*)	22·0
Smartweed-borer (*Pyrausta ainsleii*) ...	31·0

it was remarkably resistant to putrefaction, and studied the effect of enzymes such as pepsin upon it. The resistance of frog ovomucin to putrefaction was for long a puzzle to biochemists, but it seems to be explained by the unwillingness of most bacteria to grow on pure proteins, and as the jelly contains no enzymes of an autolytic character no protein breakdown products are formed, and consequently no bacterial growth takes place. This might be considered a protection of the developing embryo from bacterial attack. It is very probable, moreover, that the mucoprotein acts as a source of nourishment for the young tadpoles immediately after hatching, for they invariably attach themselves to it after they emerge from the egg-membrane, and hang on to it by their oral suckers (for histological details consult Nussbaum and Lebrun). On the other hand, development will readily proceed in the absence of the jelly, for as Hluchovski has shown it is disintegrated by exposure to ultra-violet light and may thus be removed without harming the eggs.

The swelling which takes place in the gelatinous covering when the eggs are shed into the water was studied as long ago as 1824 by Prévost & Dumas, who measured the size of the eggs at intervals after they were laid. Their table is as follows:

Hours after laying	Diameter of egg (mm.)
0	2·5
1·5	5·0
2·5	6·3
3·5	7·1
4·5	7·2
5·5	7·1
6·5	7·3

They observed that dyes would pass through the jelly as soon as it had swollen, but not before. Similar work by Wintrebert on *Discoglossus pinctus* gave the following figures:

Hours after laying	Diameter of egg (mm.)
0·00	2·5 × 2·3
0·03	3·0 × 2·7
0·16	3·3 × 3·0
0·66	5·8 × 3·2
8·00	5·0 × 4·6

As regards the mineralogical and morphological structure of the egg-shells of the lower animals, a good deal is known, and for full detail the reviews of Prenant and of Biedermann should be referred to. The majority of reptile egg-shells have their calcium carbonate in the form of calcite, as Kelly; Schmidtt, and Meigen have shown, but the two first-named investigators discovered that the egg-shells of chelonia were of aragonite, and later Lacroix observed a similar phenomenon in the case of certain saurians. The egg-

Table 33. *Elementary analyses of constituent substances of eggs.*

Species	Protein	C	H	N	S	P	O	Fe	Investigator and date
REPTILES									
Tortoise	Emydin	49·44	7·40	15·60	—	—	27·60	—	Valenciennes & Frémy (1854)
Grass-snake (*Coluber natrix*)	Keratin	54·68	7·24	16·37	None	None	21·10	—	Hilger (1873)
„ (*Tropidonotus natrix*)	Vitellin	50·64	7·84	12·03	0·20	Trace	—	—	Galimard (1904)
Crocodile (*Crocodilus biporcatus*)	Keratin	—	—	—	5·35	—	—	—	Neumeister (1895)
AMPHIBIA									
Frog (*Rana temporaria*)	Batrachiolin	—	—	16·43	—	1·36	—	None	Fauré-Fremiet & Dragoiu (1923)
„ „	The soluble fraction (37 %)	—	—	16·05	—	3·06	—	—	„ „
„ „	The insoluble fraction (52 %)	—	—	15·40	0·38	0·88	—	—	„ „
Frog	Jelly mucin	52·70	7·10	9·30	1·32	—	—	—	Giacosa (1882)
„	Batrachiolin	53·61	7·79	15·32	Trace	—	24·27	—	Galimard (1904)
„	„	—	—	15·14	1·32	1·21	—	—	McClendon (1909)
FISHES									
Trout (*Savelinus fontinalis*)	Keratin	—	—	15·82	0·8	0·14	—	—	König & Grossfeld (1920)
Cod	„	—	—	15·64	1·03	0·84	—	—	„ „
Carp	Ichthulin	53·13	7·85	15·49	0·70	0·51	22·44	0·117	Walther (1891)
Cod (*Gadus merlangus*)	„	52·44	7·45	15·96	0·92	0·65	22·58	—	Levene & Mandel (1907)
Herring	Clupeovin or ichthulin	53·68	7·38	14·64	0·40	Trace	23·90	—	Hugounenq (1904)
„	Breakdown products of ichthulin:								
	Paranuclein	47·98	7·18	14·66	0·30	2·42	27·46	—	Walther (1891) (pepsin)
	Ichthulic acid	32·56	6·00	14·03	0·15	10·34	—	—	Levene & Mandel (1907) (acid hydrolysis)
„	Ichthulin	—	—	—	—	0·73	—	—	Rewald (1929)
Carp	„	—	—	15·95	0·96	0·56	—	—	König & Grossfeld (1913)
Herring	„	—	—	15·52	1·55	1·56	—	—	„ „
Trout (*Savelinus fontinalis*)	„	—	—	15·63	0·95	0·78	—	—	„ „
Cod	„	—	—	15·99	1·15	0·71	—	—	„ „
Herring	Albumen	—	—	15·60	1·46	1·05	—	—	„ „
Cod	„	—	—	15·44	1·28	0·34	—	—	„ „
Ray (*Raia clavata*)	Ichthin	51·00	6·70	15·00	—	1·90	25·40	—	Valenciennes & Frémy (1854)
Carp	Ichthulin	52·50	8·00	15·20	1·00	0·60	22·70	—	„ „
Perch	„	52·27	6·84	14·84	1·16	0·61	—	—	Hammarsten (1905)
„	Mucin	49·09	6·69	13·04	1·54	—	—	—	„
„	"Pseudonuclein", a breakdown product of ichthulin	—	—	—	1·02–1·31	1·70–3·24	—	—	„
Cod (*Gadus aeglefinus*)	Ichthulic acid	—	—	14·24	—	8·35	—	—	Levene & Mandel (1907)
„ „	Ichthulin	52·44	7·45	15·96	0·92	0·65	22·58	None	Levene (1901)
„ „	Ichthulic acid	32·56	6·00	14·03	0·15	10·34	—	—	„
Hen (for comparison)	Vitellic acid	32·31	5·58	13·13	0·33	9·88	—	—	Levene & Alsberg (1900)

Carp	Ichthulin	53·52	7·71	15·64	0·41	0·43	22·19	0·10	Walther (1891)
,, ...	Ichthulic acid	47·75	7·15	12·70	—	2·85	29·30	0·25	,,
,, ...	"Casein paranuclein" ...	48·00	6·65	14·20	—	2·07	—	0·75	Lubavin (1870)
Torpedo (*Torpedo marmorata*) ...	Ichthulin	—	—	15·67	—	—	—	—	Rothera (1904)
Sturgeon	,, ...	—	—	16·09	—	—	—	—	,,
Carp	,, ...	52·60	7·74	15·15	0·90	0·37	25·50	—	Gobley (1850)
Dogfish (*Scyllium stellare*) ...	Keratin	53·92	7·33	15·08	1·44	—	—	—	Buchtala (1908)
,, (*Pristiurus melanostoma*)	,, ...	51·45	6·61	14·33	1·52	—	—	—	,,
,, (*Scyllium canicula*) ...	,, ...	53·64	6·49	14·23	1·33	—	—	—	,,
,, (*Scyllium catulus*) ...	,, ...	51·50	6·51	15·34	0·88	—	—	—	Krukenberg (1886)
,, (*Squalus acanthias*) ...	"Yolk-globulin"	50·55	7·18	16·71	0·91	None	—	None	Alsberg & Clark (1908)
,, ,, ...	Ichthulin	48·80	7·58	14·75	1·17	0·62	—	—	Needham (1929)
,, ,, ...	Thuichthin	45·60	7·39	12·14	1·96	0·039	—	—	,,
Herring	Ichthulin	52·39	7·58	14·09	0·89	0·014	—	None	Steudel & Takahashi (1923)
,, ...	Keratin	51·52	7·79	—	0·55	0·07	—	—	Steudel & Osato (1923)
Trout (*Savelinus fontinalis*) ...	,, ...	—	—	15·82	0·80	0·14	—	—	König & Grossfeld (1913)
Cod	,, ...	—	—	15·64	1·03	0·84	—	—	,, ,,
Average from all fishes tried ...	Ichthulin	—	—	15·70	1·18	0·89	—	—	,, ,,
,, ,, ...	Albumen	—	—	15·52	1·37	0·44	—	—	,, ,,
Herring	Ichthulin "protamine" ...	—	—	15·00	—	—	—	—	,, ,,
Carp	,, ,, ...	—	—	8·60	—	—	—	—	,, ,,
Herring	Albumen ,, ...	—	—	11·80	—	—	—	—	,, ,,
Trout	Ichthulin	—	—	14·28	—	0·57	—	—	Fauré-Fremiet & Garrault (1922)
Carp	,, ...	—	—	14·82	—	0·43	—	—	,, ,,
Dogfish (*Squalus acanthias*) ...	Ichthulin i	49·57	7·12	14·37	0·57	1·42	26·96	—	Zdarek (1904)
,, ,, ...	Ichthulin ii	44·03	6·70	16·29	1·03	2·74	29·21	—	,,
,, ,, ...	Ichthulin iii	53·88	6·78	12·06	2·66	0·15	24·47	—	,,
,, ,, ...	Ichthulin	—	—	—	—	2·5	—	—	Miescher (1872)
,, ,, ...	Lecithin	—	—	2·11	—	3·43	—	—	Ponce (1924)
Salmon	Ichthulin	—	—	—	—	0·74	—	0·022	Paton (1898)
,, ...	Ichthulic acid	—	—	—	—	3·70	—	0·064	,,
Pollack (*Pollachius brandtii*) ...	Keratin	51·24	8·41	14·57	0·67	—	25·11	—	Miyake & Tadokoro (1911)
Tunnyfish	Ichthulin	—	—	13·31	—	9·36	—	—	Dezani (1908)
INSECTS									
Silkworm (*Bombyx mori*) ...	Egg-membrane chorionin ...	49·63	—	15·64	—	—	—	—	Farkas (1903)
,, ,, ...	,, ,, ...	47·27	6·71	16·93	3·67	—	24·72	—	Tichomirov (1882)
,, ,, ...	,, ,, ...	50·90	7·10	17·20	4·38	—	19·33	—	Verson (1884)
MOLLUSCS									
Octopuses (*Sepia* and *Loligo*) ...	Egg-membrane keratin ...	49·70	6·96	10·75	—	—	—	—	von Fürth (1902)
Periwinkle (*Murex truncculatus*) ...	,, ,, ...	52·91	7·61	16·09	0·47	—	23·00	—	Engel (1890)
,, ,, ...	,, ,, ...	50·88	6·81	17·88	—	—	23·97	—	Krukenberg (1885)
Whelk (*Buccinum undatum*) ...	,, ,, ...	50·72	6·82	17·92	—	—	24·54	—	,,

membranes of snake's eggs which show all variations as to lime-content (see Table 9) are, as Kelly has shown, composed of amorphous and unstable calcium carbonate. The eggs of gastropods, such as *Helix*, *Ampullaria*, *Bulimus*, *Amphidromus*, etc., are, as Turpin (*Helix aspersa*) and Rose (*Helix pomatia*), besides the workers mentioned above, have demonstrated, like those of birds in having their lime in the form of calcite. For a general theory explaining these differences see the paper of Prenant.

The shells of eggs may also contain calcium phosphate. In the hen and in birds generally there is very little, but the globules seen in their egg-shells are believed to be calcium phosphate, though no analysis has given a figure of more than 1 per cent. of this salt. In other eggs, however, there may be more; thus Gmelin found 7·3 per cent. in the egg-shells of a tortoise, and Kelly noted its presence also in those of *Bulimus* and *Lophohelia*, though she gives no analytical figures.

It is interesting to note that the mineralogical form of lime in the egg-shell may vary during the development of the embryo; thus Kelly says that the shell of many full-grown mollusca is conchite, while that of their respective embryos and eggs is calcite. Kelly found that the organic substance was a remarkably constant proportion of the shells of mollusca, reptilia and birds (see Table 9). Some egg-coverings contain almost no water at all (birds), others have more than the egg-contents, as has been shown for the trout's egg by Kronfeld & Scheminzki (membrane 75 per cent., egg 66 per cent.).

By far the commonest substance of which egg-membranes are composed is keratin, though this protein seems to take many forms, and not to have exactly the same properties in different situations. The earlier workers were content to assert the presence of it on the basis merely of solubility tests. Thus in 1874 Schenk studied the egg-shell of *Raia quadrimaculata*, and decided that it was 95 per cent. keratin after the application to it of the protein colour reactions and an examination of its behaviour towards various solvents. The same conclusion was arrived at by the same methods by Hussakov & Welker for the egg-cases of *Raia erinacea*, and the Port Jackson shark, *Heterodontus philippi*. The keratin of these egg-cases was insoluble in all solvents except acid and alkali. They found that sulphur was present, but no phosphorus, and they were unable to find any reducing sugar after total hydrolysis. Irvine, using an optical test for chitin, found none in elasmobranch egg-cases. Krukenberg in 1885 decided that the egg-case of *Scyllium stellare* was of a keratinoid nature, because of its percentage composition, in which he found a marked amount of sulphur. He observed the interesting fact that the egg-cases of this fish, while still in the uterus of the parent animal, would dissolve in pepsin and trypsin, while after they were laid they would not dissolve in solutions of either enzyme. He also isolated tyrosine and leucine from the keratin of the egg-cases of *Scyllium stellare*. He made very similar researches on the egg-cases of *Scyllium canicula* and *Myliobatis aquila*, finding that they possessed rather different properties and seemed to be of different constitution; thus on hydrolysis he recovered a great deal of leucine and hardly any tyrosine from the keratin of *Scyllium canicula*, while from the keratin of *Myliobatis* the yields were precisely reversed. The latter substance was also considerably more resistant to digestion than the former, and Krukenberg considered that the former was not a keratin at all. He had already decided (wrongly, as it turned out) that the shell-membrane of the hen's egg was mucin, not keratin, and now he concluded that this also applied to the egg-case of *Scyllium stellare*, as well as to that of *Loligo vulgaris*, of which he made a separate examination. He thought it possible also that the jelly which surrounds the egg in the ovoviviparous selachians might be a mucin too, especially as, according to Schenk, it was not precipitated by chromic acid, and he himself found that it was extremely resistant to digestion by enzymes. This material has received no further chemical investigation since the time of Krukenberg.

Other workers who identified the proteins of egg-membranes by the aid of colour tests and solubility reactions were Leuckart, who showed, as far as anything could be shown with such preliminary methods, that the membranes of planarian eggs were of chitin, and Yoshida & Takano and Jammes & Martin, who drew a similar conclusion about the coats of the eggs of *Ascaris lumbricoides*, which they found were readily soluble in gastric juice or in any acid.[1] The case of the parasitic nematodes is of special interest, for the chitinous membrane does not arise until after the fertilisation of the egg, being, therefore, in a sense, analogous to the fertilisation membranes of echinoderms. Whether the chitin is formed as it is required during these early stages, or whether it is already present in the unfertilised egg-cell in some soluble form, is uncertain. Fauré-Fremiet in an attempt to throw light on this question, prepared pure samples of chitin from the newly fertilised eggs of *Ascaris megalocephala* by boiling them with strong potash, and identified the chitin chemically, isolating glucosamine hydrochloride from it. Remembering that Weinland showed that chitin is probably formed from glycogen during insect metamorphosis, Fauré-Fremiet estimated the glycogen in the *Ascaris* eggs before and after fertilisation. Before fertilisation there was an average amount of 20 gm. per cent. dry weight, but afterwards only 4·67, the extreme values being 5·91 and 3·23, so that no less than 17 per cent. of glycogen had disappeared. Estimations of chitin in the egg-envelopes after fertilisation gave results of between 8·3 and 10·7 per cent. dry weight of glucosamine (calculated as glycogen) with an average of 9·23. The total glucose, then, in the fertilised eggs was 12·83 to 15·08, as against 20·0 in the unfertilised ones, a loss of 7 to 9 per cent. All the glucose lost, therefore, could not have transformed itself into chitin, but must have had some other destination, perhaps butyric and valerianic acid if Weinland's view is correct. The eggs of *Ascaris* have also an "ovospermatic membrane", but for the discussion of the significance of this reference should be made to the memoir of Fauré-Fremiet, and nothing is known about it chemically. Their third membrane, the internal one, would seem to be composed to a large extent of ascaristerol (see p. 352), for the histological evidence demonstrates a collection of the ascaristerol globules at the periphery of the cytoplasm. After fertilisation, Fauré-Fremiet found the saponification number of ascaristerol lowered from 199 to 145, from which he concluded that its constitution had been slightly altered. Zavadovski has also described the egg-shells of many nematodes.

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[1] See also Campbell on the chitin of insect egg-membranes.

Neumeister, who found more than 5 per cent. of sulphur in the shells of the reptiles, *Calotes jubatus*, *Ptychozoon homalocephalus*, and *Crocodilus biporcatus*, concluded that they consisted of a true keratin, and the reactions given by the egg-membrane protein of a monotreme, *Echidna aculeata*, led him to the same conclusion in that case also. Table 9 gives the figures which he obtained for the calcium and other constituents of some of these egg-shells, as well as the very similar investigations of Wicke & Brummerstadt on *Alligator sclerops*. From these fragmentary results, it would seem that the egg-membrane protein is here keratin, and a quantity of calcium is secreted into the membrane by the animal, varying in amount from

90 per cent. to 10 per cent., according to the species. Again, the egg-membrane of the Brazilian gastropod studied by Roffo & Correa is said, on the basis of qualitative tests only, to be a true keratin, containing no reducing sugar and associated with no other substances, save 2·45 per cent. of ash. It contained calcium the amount of which did not vary during development.

The transparent horny egg-membrane of the selachian *Mustelus laevis*, which disappears half-way through the development of the embryo, has also been investigated by Krukenberg, who compared it with the egg-membrane of the grass-snake, *Tropidonotus natrix*. The former resembled the shell-membrane of the hen's egg rather than the true keratin of the *Myliobatis* egg-case. The latter seemed to have some of the properties of elastin and some of those of keratin; from it he was able to isolate a reducing carbohydrate as well as glycine, tyrosine and leucine.

Krukenberg was also one of the earliest workers to make quantitative investigations on this subject. His figures for the protein of the egg-shells of *Murex trunculatus* and the whelk *Buccinum undatum*, which are given in Table 33, led him to make a new class of such substances, the conchiolins. As no data exist for the sulphur content of most of these proteins, it is impossible to say whether they are keratins or not, and the whole subject needs re-investigation. About five years later, Engel also investigated the egg-membrane protein of *Murex*, and, obtaining 0·5 per cent. of sulphur from it, concluded, its other properties taken into account, that it was a keratin. Engel also agreed with Hilger, whose figures for the egg-membrane of the snake, *Coluber natrix* (see Table 30), suggested an elastin as its principal component. He had not been able to find any sulphur in it. About the same time, Wetzel examined the conchiolin in the egg-shells of *Mytilus edulis*, and obtained from it, after hydrolysis, leucine, tyrosine, glycine, various hexone bases and ammonia, but no phenylalanine.

The first efforts at quantitative discrimination between egg-membrane proteins were contented with ascertaining the elementary composition; thus von Fürth analysed the protein of *Loligo vulgaris* eggs in this way (39 per cent. glucosamine), and Verson, and later Tichomirov, decided that the egg-shell of the silkworm, *Bombyx mori*, was a keratin-like body (3·7 per cent. of sulphur), though, owing to its unusual properties, they called it chorionin. Of these two last-named analyses, it is probable that Tichomirov's is the more accurate, for he was more careful to remove all the adhering silk than was Verson, and Farkas' independent work agrees rather with his. It is, at any rate, clear that the shell-substance of the silkworm's egg is not chitin. According to Lavini the inorganic constituents of the silkworm egg-shell are potassium silicate, sulphate, and carbonate, to the exclusion of all other salts.

The work of Pregl and of Buchtala in 1908 is perhaps the most thorough investigation of the amino-acid distribution of an egg-membrane protein. The figures they obtained are given in Table 34. The keratin of the egg-case of *Scyllium stellare* was the only one of which they made a complete amino-acid analysis; for that of *Pristiurus melanostoma* and *Scyllium canicula* they only determined the cystine content and large groups such as the monoamino-acid nitrogen. *Scyllium* ovokeratin seemed to follow very closely in its constitution the ovokeratin of the hen, according to the figures of Abderhalden & Ebstein, which have already been discussed, but separated itself off very sharply from it on account of its high tyrosine content. The ovokeratin of the tortoise *Testudo graeca*, which had been investigated two years previously by Abderhalden & Strauss, was again different, having no tyrosine, but a very high percentage of proline. As far as this work goes, it would seem right to conclude that, though the eggs of different species may use similar proteins in their external membranes, the constitution of these proteins may vary very considerably.

The work of Steudel & Osato, and of Osato, however, brought a new factor into the problem. Their analyses of the egg-membrane protein of the herring's egg, which are shown in Tables 34, 38 and 39, gave results which differed from the usual keratin figures, but which very closely approached the analyses which they were making at the same time of the ichthulin of the herring's egg. Thus the amide nitrogen (2·05 per cent.) was lower than any of the keratins, but approximated instead to the 1·81 per cent. of herring ichthulin. What appeared to be the case on a general survey turned out to be certainly so when the amino-acid distribution was examined, for the two sets of figures almost exactly corresponded. The properties of the egg-membrane protein and the minute amount of sulphur in it precluded its classification as a keratin, and the fact that no reducing sugar could be discovered among its breakdown products was convincing evidence against its being a mucin. Osato suggested that it was

Table 34. *Distribution of amino-acids in egg-proteins.*

	Reptiles		Amphibia		Fishes																Insects	Molluscs		Echinoderms	
	Abderhalden & Strauss: ovokeratin of tortoise (*Testudo graeca*)	Galimard: vitellin of grass-snake (*Tropidonotus natrix*)	Gortner: mixed egg-proteins of salamander (*Cryptobranchus alleghamensis*)	Galimard: vitellin (ranovin) of frog	Pregl & Buchtala: ovokeratin of dogfish (*Scyllium stellare*)	Buchtala: ovokeratin of dogfish (*Pristiurus melanostomia*)	Buchtala: ovokeratin of dogfish (*Scyllium canicula*)	Steudel & Takahashi: ichthulin of herring	Steudel & Osato: egg-membrane protein of herring	Miyake & Tadokoro: ovokeratin of pollack (*Pollachius brandtii*)	Dezani: ichthulin of tunnyfish	Hugounenq: ichthulin of herring (clupeovin)	König & Grossfeld: ichthulin of trout	König & Grossfeld: ichthulin of cod	König & Grossfeld: ichthulin of sturgeon	König & Grossfeld: albumen of cod	König & Grossfeld: albumen of sturgeon	Iguchi: ichthulin of herring	Osato: egg-membrane protein of herring	Gortner: mixed egg-proteins of trout (*Savelinus fontinalis*)	Tomita: egg-membrane protein of *Bombyx mori*	Komori: mucoprotein of gastropod (*Hemifusus tuba*)	Komori: vitellin of gastropod	Russo: mixed egg-proteins of *Strongylocentrotus lividus* coagulable by acetic acid	Russo: mixed egg-proteins of *Strongylocentrotus lividus* not coagulable by acetic acid
Amide N ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6·2	7·9
Glycine	Present	—	—	—	2·6	—	—	—	—	—	—	None	—	—	—	—	—	None	None	—	13·72	—	None	—	—
Alanine	—	—	—	—	3·2	—	—	—	—	—	—	Present	—	—	—	—	—	0·3	0·31	—	3·80	—	0·71	—	—
Valine	—	—	—	—	—	—	—	—	—	—	—	Present	—	—	—	—	—	2·3	2·56	—	0·28	—	0·27	—	—
Leucine	Present	19·40	—	13·20	5·8	—	—	—	—	2·4	—	21·2	2·5	2·6	7·1	4·1	8·8	9·2	9·04	—	1·46	9·5	10·29	—	—
Proline	11·8	—	—	—	4·4	—	—	—	—	—	—	—	—	—	—	—	—	0·47	Present	—	2·17	—	1·10	—	—
Phenylalanine ...	Present	—	—	—	3·3	—	—	—	—	—	—	Present	—	—	—	—	—	—	—	—	0·69	—	0·22	—	—
Aspartic acid ...	1·2	—	—	—	2·3	—	—	—	—	—	—	Present	—	—	—	—	—	—	—	—	0·37	—	1·60	—	—
Glutamic acid ...	2·9	—	—	—	7·2	—	—	—	—	1·4	—	None	—	—	—	—	—	None	0·35	—	4·16	—	None	—	—
Serine	—	—	—	—	—	—	—	—	—	—	—	Present	—	—	—	—	—	—	—	—	1·10	—	None	—	—
Tyrosine	None	0·60	—	1·03	10·6	—	—	—	—	1·53	—	1·0	2·8	5·4	5·5	1·8	8·6	3·8	3·66	—	11·19	0·1	0·80	—	—
Cystine	—	—	0·21	—	0·44	0·60	0·42	—	—	—	—	—	—	—	—	—	—	0·93	0·62	0·34	None	—	—	1·8	0·8
Histidine	—	0·30	5·63	1·14	1·7	—	—	2·45	3·99	Trace	2·23	0·4	0·54	0·55	0·47	0·31	0·82	—	—	8·83	Present	None	None	3·8	13·1
Arginine	—	0·32	13·56	1·06	3·2	—	—	14·50	14·41	2·3	0·95	2·7	0·41	0·54	0·97	0·44	0·92	—	—	11·09	0·19	0·55	3·73	10·8	2·4
Lysine	—	1·45	10·16	0·29	3·7	—	—	10·07	7·51	1·75	13·53	2·0	0·01	0·02	0·01	0·03	0·07	—	—	8·33	0·39	0·22	0·86	15·2	1·9
Ammonia	—	—	9·96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·81	2·05	7·56	—	—	—	—	—
Tryptophane ...	—	—	—	—	Present	—	—	—	—	—	—	—	—	—	—	—	—	—	1·77	—	—	—	1·49	—	—
Humin	—	—	2·25	—	—	—	—	—	—	—	—	22·0	—	—	—	—	—	—	—	1·82	—	—	—	—	—
Unidentified ...	—	77·93	—	73·28	—	—	—	—	—	—	—	50·7	—	—	—	—	—	—	—	—	—	—	—	—	28·4
Total di-amino N ...	—	—	—	—	—	—	—	—	—	—	16·71	—	—	—	—	—	—	—	—	—	—	—	—	29·8	17·3
Total mono-amino N	—	—	53·73	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	61·55	—	—	—	62·2	45·7
Non-amino N of mono-amino fraction	—	—	2·76	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3·90	—	—	—	—	—

simply an insoluble modification of ichthulin. As he pointed out, industrial use has long been made of insoluble forms of proteins, such as casein, and there was no reason why the egg-membranes of certain eggs, at any rate, should not be insoluble modifications of the proteins of their yolks. Steudel & Osato also suggested that the ovomucoid of the egg-white of the hen might be a phylogenetic reminiscence of the mucoprotein with which the amphibian egg is surrounded. For a review of this work see Steudel.

The eggs of salps and tunicates are surrounded by a coat of very much smaller cells which act as some sort of protection for the developing embryo inside. Zavattari has demonstrated histochemically the presence of an abundance of glycogen in these test cells, and believes that they have a nutritive function. If so, this would be a third case where such an active participation of the shell or case in embryonic metabolism would have been noted, the two others being the abstraction of calcium from the shell of the hen's egg, and the contribution of amino-acids by the egg-case of the silkworm.

A good deal is known about the osmotic and other properties of the membranes of amphibian and fish eggs, but these are so intimately associated with the physico-chemical processes taking place during development that consideration of them will be postponed to Section 5. It will suffice to mention here the experiments of Peyrega, who found that the egg-cases of *Scyllium canicula* were permeable to salt. He fitted up osmometers with small pieces of the case as the membranes, and observed that it took about 20 days to establish osmotic equilibrium with respect to solutions of sodium chloride about as strong as sea water, when distilled water was put on the other side. These egg-cases have also been shown by Needham & Needham to be permeable to urea and ammonia.

1·13. Proteins and other Nitrogenous Compounds

The principal protein substance which is found to occur in the eggs of all known animals closely resembles the vitellin of the hen's egg. It has even been found, according to Chatton, Parat & Lvov, in the food-reserves of infusoria. The early analyses of the eggs of the pike by Vauquelin in 1817, of the barbel (*Cyprinus barbus*) by Dulong d'Astafort in 1827, and of the trout (*Salmo fario* and *Cyprinus carpio*) by Morin in 1823, led to no more than the view that an albuminous substance was present in them. But with the work of

Gobley on the hen's egg, which has already been described, a more solid basis for comparison was achieved, and Valenciennes & Frémy, in a memoir which received a prize from the Academy of Sciences and which was translated into English, proceeded to examine the eggs of as many species as were available to them. Gobley's only excursion into comparative chemical embryology had been a detailed analysis of the carp's egg, published in 1850, but he had not been slow to point out the differences between this analysis and that of the hen's egg. His figures are shown in Tables 2, 30 and 33, where it will be seen that he got a value of 15·76 per cent. protein (wet weight) for the hen, and 14·23 per cent. for the carp, but 31·43 per cent. fat for the hen and only 2·57 per cent. fat for the carp. The carp's egg had, he found, about 10 per cent. more water than the yolk of the hen's egg, but only a third of the lipoid substances.

Frémy & Valenciennes specially directed their attention to the protein fraction, and attempted to discover whether the vitellin was the same in all eggs. For the most part they relied on histological appearances (the "dotterplättchen" were greatly discussed at this time), but they also examined the solubility relationships of the proteins from each egg, and in some cases subjected the purified substances to elementary analysis. The figures they obtained for the different compounds are all given in Table 33, and the eggs they investigated in Table 35. They were able to isolate a number of vitellin-like proteins, soluble in salt solution and precipitated by the addition of water. They compared vitellin with fibrin, and concluded that the two substances were almost identical, in spite of slight differences in the analytical figures—"for bodies of this nature", they said, "which are not crystallisable and insoluble in water and which are therefore very difficult to purify, where is the chemist who could answer for 1 per cent. of nitrogen in an elementary organic analysis?" Ichthin, which they isolated from fish eggs, differed from vitellin by not becoming an opaque mass when placed for a long time in boiling water, and by giving a violet instead of a blue colour when treated with boiling hydrochloric acid. Ichthidin, another product of fish eggs, differed from ichthin in being soluble in water. Ichthulin, the third member of the group, differed from the others in not being soluble in all dilutions of saline, but in being precipitated from the aqueous extract by further addition of water. As for emydin, it closely resembled ichthin, and it is not easy to see why Valenciennes & Frémy did not identify it with that substance. The remaining egg-proteins, which they did not further investigate, they referred to under the generic name of albumen.

Table 35. *Investigations of Valenciennes & Frémy.*

AVES	*Gallus domesticus*		Vitellin
PISCES			
Elasmobranchs	*Raia clavata*		Ichthin
	Torpedo marmorata		,,
	Scyllium canicula		,,
	Galeus canis		,,
	Mustelus laevis		,,
	Squatina angelus		,,
	Raia fullonica		,,
	Raia rubus		,,
Teleosteans	*Cyprinus carpio*		Ichthidin and ichthulin
	Labrax lupus		Ichthulin and ichthidin
	Mugil chelo		,,
	Scomber scombrus		,,
	Pleuronectes maximus		,,
	Pleuronectes solea		,,
	Solea armorica		,,
	Unidentified species of	salmon	,,
	,,	eel	Albumen
REPTILIA	*Testudo mauritanica*		Emydin
	Cistudo europaea		,,
	Unidentified species of	lizard	Vitellin
	,,	grass-snake	,,
	,,	viper	,, (?)
AMPHIBIA	,,	frog	Ichthin
	,,	newt	,,
CRUSTACEA	,,	lobster	Albumen
ARACHNIDA and INSECTA	—	—	Albumen
MOLLUSCA	—	—	Not albumen

The differences between the compositions which Valenciennes & Frémy found for these substances are not great, and it is very doubtful whether they are more than modifications of the same substance, especially as these workers admittedly had great difficulty in obtaining pure preparations. But the problem of the identity of the vitellins is not yet settled. The later investigations are all grouped together in Table 33, and the differences between the preparations can easily be seen to be small. The work of Plimmer & Scott proved that ichthulin is a phosphoprotein closely allied to vitellin. Among the more interesting observations must be mentioned those of Levene & Mandel; Levene, and Walther, on ichthulic acid obtained from the ichthulins of various fish eggs by digestion with pepsin and other methods. These with their very high phosphorus content approach closely the "paranucleins" or vitellic acids obtained from the vitellin of the hen's yolk by Levene & Alsberg and others. Evidently there are several possible stages of breakdown, for Walther's ichthulic acid only contains 2·8 per cent. of phosphorus, while that of Levene has as much as 10·4. Here, also, however, there are great variations; thus, while nearly all the ichthulins studied have from 0·6 to 1·9 per cent. of phosphorus, the preparation of Steudel & Takahashi from the herring's egg has only 0·014 per cent. In the yolk of a dogfish egg, Zdarek found no less than three proteins, the third of which may possibly correspond with König & Grossfeld's albumen class.

In 1908 Alsberg & Clark claimed that phosphorus was quite absent from the principal protein of the egg of an ovoviviparous selachian, *Squalus acanthias*, but some twenty years later I re-examined the question and obtained without difficulty 0·6 per cent. from selachian ichthulin (derived from the same species). This yolk also contains a second protein, thuichthin, corresponding closely in properties and constitution with the ovolivetin of the hen studied by Kay & Marshall (see Tables 10 *a* and 33).

Gray has studied the properties of the ovoglobulin or ichthulin of *Salmo fario*. If the yolks are poured into water, a dense white clot is formed and the water becomes cloudy. The precipitate is soluble, however, in acids, alkalies and neutral salts. When the egg-cell dies, the egg becomes opaque, and this must certainly be due to the precipitation of the globulin, for by placing dead white eggs in normal sodium chloride solution they rapidly become clear and resemble normal eggs, but regain their opacity when removed to distilled water. The clearing process takes 15 minutes but the precipitation takes 1½ hours. Evidently the dead protoplasmic membrane can no longer retain in the egg the electrolytes necessary for solution of the ichthulin.

Further work on the properties of teleostean ichthulin was done by Runnström. 1·5 parts of egg "Pressaft" having been added to 1·28 parts of water and the ichthulin precipitated, the effect of various ions on its solubility was tried. The anions placed themselves in the order:

$$SCN > I > NO_3 > SO_4 > Cl > \text{acetate}.$$

Thus for 2 c.c. of potassium chloride solution, 0·3 c.c. of distilled water had to be added to get coagulation, but to 2 c.c. of KSCN solution, as much as 6·4 c.c. The cations went as follows:

$$\mathrm{Ca} > \mathrm{Mg} > \mathrm{Sr} > \mathrm{K} \text{ and } \mathrm{Na}.$$

The egg-white of the dogfish egg was thought by Brande in 1810 to be identical with the jelly surrounding the egg of the frog, but whether the former really consists of mucin and not albumen cannot be definitely stated, for no work has since been done on it. However, my wife and I, in our work on the eggs of *Scyllium canicula*, frequently observed a coagulation of the egg-white with acetic acid, which would point to the latter possibility.

The proteins of the echinoderm egg have never been properly investigated. Vlès, Achard & Prikelmaier have estimated from cataphoresis experiments that the average isoelectric point of the *Paracentrotus lividus* egg-proteins lies between 5·0 and 5·8 *p*H, but their grounds for this figure are not free from criticism.

Vlès & Gex, in some interesting experiments, have studied the normal unfertilised sea-urchin's egg spectrophotometrically. The absorption spectrum of the normal egg has peaks or bands at wave-lengths of 490, 395, 370, 315, and 230 Ångström units, and a marked trough between 260 and 240 λ. This curve is very peculiar, for on the one hand it shows much transparency in the ultra-violet although most organic substances do not, while on the other hand there is nothing at all corresponding to the bands of absorption about λ 275 which all proteins give. This absorption is brought about by the cyclic amino-acids in the protein molecule, and it is quite impossible that these should be altogether absent from the egg-proteins of the sea-urchin. Vlès & Gex considered various technical possibilities which might explain these effects, but did not think that any of them would account for what was perhaps the most remarkable part of the investigation, namely, the finding that on cytolysis ("white") a perfectly definite and clear absorption spectrum for protein revealed itself. In the intact egg, then, this must be masked by something else. Speculation on the nature of this mechanism would be easy, for all kinds of effects might be responsible, e.g., formation of complexes, reduction equilibria, and satisfaction *in vivo* but not *in vitro* of residual valencies in the protein molecule. If this very interesting work should lead in the future to a revivification in a subtler form of the old biogen molecule theory (though it is to be hoped that it will not), not only as regards the egg-cell but as regards protoplasm in general, we shall at any rate possess in the spectrophotometer a powerful means of studying the untouched normal cell-interior.

Doubt exists with respect to the presence of reducing carbohydrate in the ichthulin molecule. Levene & Mandel obtained minimal quantities of laevulinic acid from their cod ichthulin, but this finding was associated with the presence of purine bases. Six years earlier Levene had been unable to find a trace of glucosamine in cod ichthulin. Similar negative results were obtained by Steudel & Takahashi on herring, and by Hammarsten on perch, ichthulin. But the presence of glucosamine in notable amounts has been reported for *Torpedo* ichthulin by Rothera, and for carp ichthulin by Walther. While it is possible, and even probable, that ichthulins from different fish eggs may vary much, it would be very desirable to know to what extent this is the case, and a comparative study of ichthulins is much needed. As we have seen Levene & Mori have isolated a trisaccharide from avian vitellin.

Closely allied to the question of the presence of carbohydrate groupings in the ichthulin molecule is the equally disputed problem of the presence of purine bases in the undeveloped egg. We have already seen that Miescher's identification of nucleoprotein with vitellin was quite erroneous, and have described how he was set right by Kossel. For the hen's egg, it is now fairly clear that nucleins are present only in exceedingly small amounts at the beginning of development, not exceeding, for instance, 1 or 2 per cent. of the total nitrogen or phosphorus. But there has been more difficulty in deciding what is the real state of affairs in the eggs of fishes and aquatic invertebrates. Walther (carp), Hugounenq (herring), Linnert (sturgeon), and Hammarsten (perch), all examined the ichthulin of these eggs for nucleic acid, and all failed to find the least trace of it. Henze, on the other hand, working with the whole egg of the cephalopod, *Sepia officinalis*, isolated considerable amounts of purines together with no less than 1·15 gm. per cent. of a pentose. Tschernorutzki a little later found that 10 per cent. of the total phosphorus of the herring's egg could be accounted for as nucleoprotein phosphorus, and the nucleoprotein itself amounted to 1·19 gm. per cent. dry weight. Masing; Tichomirov, and Needham & Needham reported quite similar results with the sea-urchin's egg, the egg of the silkworm and the eggs of various crustacea, echinoderms and an annelid. In the sea-urchin egg purine bases were found accounting for 6 per cent. of the total nitrogen as nucleoprotein nitrogen, while in the case of *Bombyx* there were 20 mgm. per cent. dry weight. Again, Levene & Mandel isolated from their ichthulic acid in 1907 0·344 per cent. of guanine, 0·307 per cent. of adenine, 0·360 per cent. of uracil and 0·309 per cent. of thymine. Mandel & Levene were also able to isolate nucleic acid from cod's eggs. It would certainly appear from this evidence as if ichthulin and vitellin may be associated with small quantities of nucleic acid. In this connection it is of interest that Calvery has evidence that the chick embryo can synthesise "yeast-" as well as animal nucleic acid. Steudel & Osato have also obtained guanine and adenine from herring's eggs, but this was in the non-protein nitrogen fraction, and there was therefore no evidence from their work that any preformed nucleic acid was a constituent of the egg. The most exhaustive investigation of the problem was that of König & Grossfeld, who in 1913 set out definitely to clear up the discrepancy. As perhaps might have been expected, they found that they could isolate purine bases after hydrolysis from all the fish eggs they studied, but only in small quantity; their results are shown in Table 36. The question of nuclein synthesis by the developing embryo will be discussed in relation to these findings in Section 10·3.

Table 36. *Investigations of König & Grossfeld.*

	Total purine bases isolated Mgm. % dry weight
Herring	0·408
Carp	1·060
Cod	2·440
Pike	0·014
Sturgeon	0·230

But the exact relationship between the nuclein and the vitellin remains exceedingly obscure. It is possible that in one and the same egg there may be more than one modification of vitellin, apart altogether from the insoluble form suggested by Steudel & Osato. All the knowledge that we possess at the present time on this point is of an unsatisfactory histological nature, and any discussion of it must inevitably include an unprofitable proportion of guesswork. Thus, Jörgensen differentiated histologically between two substances which seemed to be present in the unripe egg of *Patella vulgata*, ergastoplasm No. 1 and ergastoplasm No. 2, one at least of which was responsible for the formation of the vitelline globules. Fauré-Fremiet & Garrault identified ergastoplasm No. 1 with the mitochondria, and ergastoplasm No. 2 with the fatty constituents of the yolk. But if two forms of vitellin existed, one in loose combination with a nuclein and the other free, the staining reactions of histological elements mainly constituted by one or other of these substances would very likely differ, and it is possible that an explanation on these lines may in the future correlate the chemistry with the histology of the yolk. The vitellin question has been in a measure reviewed by McCrudden, whose table (given in Table 37) illustrates the difficulty of summing up the findings of investigators at all succinctly.

Table 37.

Species	Protein	Iron	Phosphorus	Millon test	Tryptophane	Sulphur	Glucosamine	Investigator and date
Pike	Ichthulin	None	Much	Positive	Negative	Present	None	McCrudden (1921)
,,	Albumen	Traces	Present	,,	,,	,,	Much	,,
Barbel	Ichthulin	None	A little	Negative	,,	,,	None	,,
,,	Albumen	,,	Much	,,	,,	,,	Present	,,
Cod	Ichthulin	—	Present	—	—	,,	None	Levene (1901)
Carp	,,	Present	,,	—	—	,,	Present	Walther (1891)
Perch	,,	—	—	—	—	,,	None	Hammarsten (1905)
Salmon	,,	—	Present	—	—	,,	—	Valenciennes & Frémy (1854)
Carp	,,	—	,,	—	—	,,	—	Gobley (1850)

The amino-acid analyses (Table 34) are rather more interesting. We have data for the vitellins of the herring, the trout, the cod, and the sturgeon among fishes, the frog among amphibia, the grass-snake among reptiles, and *Hemifusus tuba*, a gastropod. To this may be added amino-acid analyses of the mixed egg-proteins of the sea-urchin egg and the eggs of the brook-trout and the giant salamander, as well as the albumens of cod and sturgeon and the mucoprotein of *Hemifusus*. If the fish ichthulin analyses of Iguchi or Hugounenq be compared with those of Table 11 for the vitellin of the hen, no very marked differences can be observed, although the predominancy of arginine and lysine over histidine, which is a constant feature of the ichthulins, reaches greater values in the latter than in the case of bird vitellin (see Table 38). Again, bird vitellin always shows a notable proportion of proline and leucine, and this is also the case with the vitellins of the lower animals (e.g. 10 per cent. of leucine in gastropod vitellin, 19 per cent. in snake vitellin and 9 per cent. in herring ichthulin), though the amount of proline is usually not so great. The only instance of a real divergence between bird and other vitellins would appear to be the glutamic acid content, which is always high in the former, although this amino-acid is absent from the latter.

Table 38. *Hexone bases of yolk-proteins.*

		In % total nitrogen			In gm. % original protein			
Species	Protein	Hist.	Arg.	Lysine	Hist.	Arg.	Lysine	Investigator and date
Herring	Ichthulin	2·45	14·50	10·07	1·28	6·33	7·40	Steudel & Takahashi (1923)
,,	Egg-membrane protein	3·99	14·41	7·51	2·09	6·35	5·55	Steudel & Osato (1923)
Hen	Vitellin	—	—	—	1·90	7·46	4·81	Osborne & Jones (1909)
Herring	Ichthulin (clupeovin)	0·40	2·70	2·00	—	—	—	Hugounenq (1904)
Sturgeon	Ichthulin	0·47	0·97	0·01	—	—	—	König & Grossfeld (1913)
Cod	Ichthulin	0·55	0·54	0·02	—	—	—	,, ,,
Trout	Ichthulin	0·54	0·41	0·01	—	—	—	,, ,,
Gastropod	Ichthulin	None	3·73	0·86	—	—	—	Komori (1926)
Frog	Vitellin (ranovin)	1·14	1·06	0·29	—	—	—	Galimard (1904)
Snake	Vitellin	0·30	0·32	1·45	—	—	—	,,

If now Table 39 is considered, it will be seen that variations are present in the general analysis of these proteins, but that they tend to cancel each other out among the groups. Thus the mono-amino-acid/di-amino-acid ratio is very constant indeed in different ichthulins, although Rothera himself considered that he was dealing with two entirely different proteins, the vitellin of the *Torpedo* egg and that of the sturgeon. It is unfortunate that Komori's examination of gastropod vitellin was confined to the estimation of the amino-acids by isolation, and did not include a van Slyke determination of the relative amounts of mono-amino and di-amino acids. In contradistinction to the ichthulins, the mixed egg-proteins studied by Russo and Gortner show more variation, though the former's values for two sea-urchin egg proteins agree well with the usual vitellin figure. Masing, however, was not able to find any phosphoprotein phosphorus in sea-urchin eggs, and Needham & Needham found only very little. It is interesting to note that the ratio is subject to large fluctuations among the keratins of the egg-cases. As for the albumens which König & Grossfeld isolated from the eggs of the sturgeon and the cod, they seem to approach in their composition, in so far as data for the hexone bases permit one to form a conclusion, the ovoalbumen in the hen's egg. The 8 per cent. of tyrosine obtained from the sturgeon ovoalbumen is, however, remarkable. The mucoprotein which Komori found around the eggs of the gastropod *Hemifusus tuba*, and which he partially analysed, is not sufficiently well characterised to be compared except roughly with the mucoprotein of the amphibian egg-jelly.

Table 39.

			In % total nitrogen						
Species	Protein	Total N	Humin N	Amide N	Melanin N	Mono-amino N	Di-amino N	Mono-amino/di-amino N	Investigator and date
Torpedo (*Torpedo marmorata*)	Ichthulin	15·67	—	8·49	1·26	60·20	25·10	2·40	Rothera (1904)
Sturgeon	,,	16·09	—	9·51	0·99	63·60	27·65	2·30	,,
Dogfish (*Scyllium stellare*) ...	Ovokeratin	15·08	—	5·09	0·56	79·66	15·78	5·04	Buchtala (1908)
,, (*Pristiurus melanostoma*)	,,	14·33	—	5·13	0·14	66·45	28·78	2·31	,,
,, (*Scyllium canicula*) ...	,,	14·23	—	4·49	0·24	64·19	30·75	2·09	,,
Hen	,,	16·43	—	6·60	0·21	72·70	20·50	3·55	,,
Herring	Ichthulin	14·09	8·49	1·81	—	61·77	27·02	2·29	Steudel & Takahashi (1923)
,, ...	Egg-membrane protein	—	7·33	2·05	—	62·11	25·91	2·40	Steudel & Osato (1923)
Sea-urchin	Mixed egg-proteins (total)	—	—	28·4		45·70	17·30	2·64	Russo (1926)
,, ...	Mixed egg-proteins (coag. only)	—	—	—	—	62·20	29·80	2·09	,,
Brook-trout	Mixed egg-proteins	—	1·82	—	—	61·55	28·25	2·18	Gortner (1913)
Giant salamander	,, ,,	—	2·25	—	—	53·73	29·35	1·83	,,
Hen	Vitellin (for comparison)	15·29	1·63	5·55	—	67·10	25·10	2·67	Plimmer (1908)

The general distribution of nitrogenous substances in the eggs of the lower animals is shown in Tables 40 and 41. Pigorini's investigation of the silkworm egg is suggestive, but his data about the different protein fractions are insufficient to enable us to form any judgment on their relation to those so well known in the bird's egg. The very large amount of mucoprotein in the silkworm ovum is certainly remarkable. In Table 41 are placed the few data which we have on the relative amounts of protein and non-protein nitrogen in different eggs, and the way the protein is divided between keratin, albumen, and ichthulin or vitellin. Clearly enough there is great variation, and a rough dichotomy into two groups, one in which the non-protein nitrogen accounts for from 14 to 35 per cent. of the total nitrogen, and one in which it only accounts for less than 10 per cent. of the total nitrogen. It is evident from the work of König & Grossfeld that all the fishes examined belong to the first of these categories, although within the group there are wide divergences, such as the minute amount of albumen apparently present in the trout's egg and the low non-protein nitrogen of the herring's egg. Good agreement is to be noted between the results of Levene and König & Grossfeld, who all worked on the cod; and, although nothing concerning the non-protein nitrogen can be gathered from the figures of Kensington and Hugounenq, their results do show general agreement as regards the partition of nitrogen among the proteins. The only reptile on whose eggs work has been done which could be incorporated in the table is the grass-snake, and there, although no non-protein nitrogen figures are available, it is interesting to note the very high proportion of keratin.

Table 40.

Silkworm (*Bombyx mori*). (Pigorini, 1923.)

In % of total protein			
Protein sol. in distilled water (albumen)	Protein sol. in 10 % salt sol. (vitellin)	Protein sol. in dilute alkalies (nucleoprotein)	Protein sol. in water but not coagulable by heat, and yielding glucosamine on hydrolysis (ovomucoid)
29·20	8·57	11·45	50·90

The second principal group, consisting of those eggs which have a relatively much lower percentage of non-protein nitrogen, contains two members, the hen and the silkworm. The former may be said with a high degree of probability to be characteristic of all nidifugous birds, and perhaps of nidicolous ones also, but whether the latter is at all representative of the centrolecithal insect eggs may be considered doubtful. The sole insect egg which has been investigated chemically, so far, is that of the silkworm, and until more evidence is available the hen and the silkworm will have to be placed together in this second group without comment. It is significant that, in the hen's case, the percentage of albumen is greater than in any other, a fact obviously referable to the large amount of egg-white present in that egg. Finally, it is of interest that the sea-urchin's egg seems to have a protein/non-protein nitrogen ratio very like that of the fishes, but situated on the low protein edge of their limits of variation.

Table 41. *Distribution of*

Species	Water	Protein (N × 6·25)	Protein (by diff.)	Protein of egg-membrane	Albumen	Ichthulin	Nitrogen (direct)	Free bases and amino-acids	Fat	Ash
	Gm. % wet weight									
Carp	66·15	27·70	29·97	3·63	16·43		4·432	9·91	2·48	1·40
Pike	63·53	28·13	33·01	3·75	2·38	17·29	4·500	9·59	1·40	2·06
Trout	63·85	27·81	30·81	1·76	0·15	24·33	4·450	4·57	3·71	1·63
Herring	69·22	26·32	25·21	3·20	4·83	13·68	4·212	3·50	4·19	1·38
Cod	72·10	23·02	24·44	2·57	2·70	11·47	3·683	7·70	1·33	2·13
Salmon	—	—	—	—	—	—	—	—	—	—
Herring	65·00	28·55	—	0·79	28·70			—	3·62	—
Sea-urchin (*Strongylocentrotus lividus*)	80·50	6·47	—	—	—	—	—	3·90	—	—
Silkworm	—	—	35·00	—	10·20	3·00	—	2·25	—	—
,, ...	66·24	22·00	—	—	—	—	3·67	0·875	—	—
Grass-snake (*Tropidonotus natrix*)	58·94	19·24	—	12·71	0·72	5·81	—	—	—	—
Cod	94·67	—	—	—	—	—	—	—	—	—
Fresh-water gar ...	53·90	26·20	—	—	—	—	—	0·138	—	—
Hen (average results) whole egg	—	—	11·81	0·45	6·13	5·23	—	0·500	—	—
Turtle (*Thalassochelys corticata*) yolk	—	—	—	—	—	—	2·91	0·033	—	—

* With 50 % mucoprotein and

Within the non-protein nitrogen fraction itself there are some fragmentary data for the distribution, as may be seen from Table 42. Unidentified compounds usually account for from 20 to 35 per cent. of the total non-protein nitrogen, and free amino-acids for approximately half of it. Among those identified by Steudel & Osato were histidine, arginine, lysine and cystine. The ammonia may vary from 4 to 25 per cent., and the purine bases from 15 to 40 per cent. As far as can be seen at present, the hen's egg seems to possess the greater part of its non-protein nitrogen in the basic fraction. The most interesting point brought out by the table is probably the significant quantity of urea shown to be present by the analyses of Steudel & Osato, amounting to no less than half of the total non-protein nitrogen, and it is possible that a good deal of the unidentified nitrogen of König & Grossfeld might be accounted for in this way. The presence of nitrogenous excretory products in the undeveloped egg, though at first sight paradoxical, is nevertheless undoubtedly a fact in the case of some aquatic organisms. The hen's egg contains hardly a trace of urea at the beginning of development but that of a selachian fish contains a good deal (see Section 9·11).

the nitrogen in eggs.

% of the total nitrogen					Gm. % dry weight (ash free)									
Protein total	Keratin	Albumen	Ichthulin	Free amino-acids	Protein (N × 6·25)	Protein (by diff.)	Egg-membrane protein	Albumen	Ichthulin	Nitrogen (direct)	Free bases and amino-acids	Fat	Ash	Investigat and date
66·9	12·1	54·8		33·1	85·37	92·37	11·19	50·64		13·65	30·54	7·64	—	König & Gr (1913)
70·96	11·35	7·21	52·4	29·04	82·75	95·93	10·90	6·92	50·25	13·08	27·87	4·07	—	,,
85·20	15·7	0·49	79·0	14·85	80·56	89·25	5·10	0·44	70·48	12·89	13·24	10·75	—	,,
86·15	12·70	9·15	54·3	13·9	89·52	85·75	10·88	16·43	46·53	14·33	11·91	14·25	—	,,
68·41	10·50	1·01	46·9	31·5	89·33	94·84	9·97	10·48	44·51	14·29	29·88	5·55	—	,,
(100·0?)	—	11·70	88·0	—	87·80	—	—	10·30	77·5	—	—	4·50	7·50	Kensington (
(100·0?)	2·7	97·3		—	—	—	—	—	—	—	—	—	—	Hugounenq
62·0	—	—	—	37·9	—	—	—	—	—	—	—	—	—	Russo (1926)
94·0	—	27·4	8·05	6·04*	—	—	—	—	—	—	—	—	—	Monzini (19 Pigorini (1
96·0	—	—	—	3·98	65·25	—	—	—	—	—	—	—	—	Russo (1922)
(100·0?)	66·0	3·74	30·2	—	—	—	—	—	—	—	—	—	—	Galimard (19
66·0	—	—	—	33·0	68·09	—	—	—	—	—	—	—	—	Levene (189
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Nelson & G (1921)
96·4	4·06	49·8	42·5	3·66	—	—	—	—	—	—	—	—	—	—
98·5	—	—	—	1·5	—	—	—	—	—	—	—	—	—	Tomita (192

chorionin in addition.

As is well known, these fishes have a special relation to this substance. In 1858 Stadeler & Frerichs isolated "kolossale Quantitäten von Harnstoff" from the organs of plagiostomes, obtaining a solid mass of urea nitrate when they added nitric acid to their final concentrates. One liver of an adult *Scyllium canicula* gave them 2 oz. of urea, and similar high figures were reported for *Acanthias vulgaris*. Teleostean fishes, however, and the cyclostome, *Petromyzon planeri*, yielded practically no urea, at any rate not more than would be present in mammalian tissues. Stadeler confirmed the selachian results on *Raia batis* and *clavata* and on *Torpedo marmorata* and *ocellata*. In 1861 Schulze repeated and confirmed Stadeler's work on *Torpedo*, and in 1888 Krukenberg published an extensive work on the subject, in which he related his unsuccessful attempts to demonstrate urea in the bodies of teleosts (*Lophius piscatorius*, *Conger vulgaris*, *Acipenser sturio*), a cyclostome (*Petromyzon fluviatilis* and *Ammocoetes*) and a cephalochordate (*Amphioxus lanceolatus*), although he found large amounts of it in the bodies of elasmobranch fishes (*Scyllium stellare*, *Mustelus vulgaris* and *laevis*, *Acanthias vulgaris*, *Squatina angelus*, *Torpedo marmorata*, *Myliobatis aquila*) and in the holocephalic *Chimaera* *monstrosa*. Particularly interesting were his experiments with eggs—he isolated considerable amounts of urea from a 5 cm. embryo of *Mustelus laevis*, and from the yolk of *Scyllium stellare* and *Myliobatis aquila* eggs, but he could find none in the surrounding jelly or "white". An egg of *Pristis antiquorum* yielded 3920 mgm. per cent. (wet weight) and a *Torpedo ocellata* egg 1740 mgm. per cent. An *Acanthias vulgaris* embryo 17 cm. long had 3360 mgm. per cent. in its muscles, 1800 mgm. per cent. in its liver, and 2640 mgm. per cent. in its unused yolk. Other work on urea in selachians was done by Gréhant and by Rabuteau & Papillon.

Table 42. *Distribution of non-protein nitrogen in eggs.*

		% of total non-protein N (including purine N)									
Species	Total non-protein N (mgm. in % dry wt.)	Bases N	Ammonia N	Free amino-acids N	Unidentified N	Urea N	Uric acid N	Creatinine N	Total amino N	Combined amino N	Investigator and date
Herring	—	19·8	—	44·3	35·9	—	—	—	—	—	König & Grossfeld (1913)
Carp	—	39·8	—	36·1	24·1	—	—	—	—	—	,, ,,
Sturgeon ...	—	25·2	13·6	55·4	18·9	—	—	—	—	—	,, ,,
Herring	2060	2·44	6·7	21·6	—	51·9	None	18·3	—	—	Steudel & Osato (1923); Steudel & Takahashi (1923)
Herring	1443	16·91	23·42	41·65	18·02	—	—	—	—	—	Yoshimura (1913)
Silkworm ...	440	—	4·44	54·30	34·60	—	—	—	61·0	6·7	Russo (1922)
Hen (aver. figures)	—	88·80	4·22	7·04	—	None	None	Trace	—	—	—
Fresh-water gar (not ripe)	299	92·00	—	4·02	—	—	—	4·0	—	—	Nelson & Greene (1921)

More light, however, was thrown on the reasons for this richness in urea when in 1897 Bottazzi working on the osmotic pressure of fish blood, found that the elasmobranchs differed fundamentally from teleosts in being isotonic with sea water.

Serum		Δ
Selachians	*Torpedo marmorata*	−2·26°
	Trygon violacea	−2·44°
Teleosteans	*Charax puntazzo*	−1·04°
	Serranus gigas	−1·03°

Bottazzi observed that the selachian osmotic pressure would correspond to some 3·9 per cent. sodium chloride but laid no emphasis on the fact that selachian blood did not contain anything like so much ash. It was left for Rodier to show that the difference was made up almost wholly by urea. Duval has since found that the salts alone would only give an osmotic pressure of Δ − 1·06°. "High blood-urea", as Smith says, "is a phyletic character of the orders Selachii and Batoidei", and its osmotic function was well shown by the reciprocal relation between salts and urea which Smith found to hold in selachian tissues and fluids.

		Blood-urea mgm. %
Smith (1929)	Selachians	
	Dogfish (*Mustelus canis*)	880
Denis (1913)	Selachians	
	Dogfish (*Mustelus canis*)	800
	Sandshark (*Carcharias littoralis*)	1000
	Skate (*Raia erinacea*)	868
,,	Teleosteans	
	Mackerel (*Scomber scombrus*)	86
	Goosefish (*Lophius piscatorius*)	40
	Flounder (*Paralichthys dentalus*)	46

In view of all these facts it is not surprising that Needham & Needham in 1928 found about 5 mgm. of urea nitrogen present in the *Scyllium canicula* egg at the beginning of development; and 888 mgm. per cent. of urea in the undeveloped *Acanthias vulgaris* egg. Gori, again, found 710 mgm. in undeveloped *Torpedo* eggs. But since urea accumulation is closely confined to elasmobranchs it is unlikely that the results of Steudel & Takahashi and of König & Grossfeld can be interpreted as being due to urea.

The presence of urea has also been reported in the undeveloped eggs of "ants and flies" (in small quantities) by Fosse. Further details would be desirable here.

There is reason to believe that nitrogenous substances other than those already mentioned are present in certain eggs. Thus Yoshimura and Poller & Linneweh isolated trimethylamine, tetramethylenediamine and choline from fresh herring eggs, and there is a certain probability that fish eggs also contain betaine. As the characteristic smell of fish is due to these amines and related substances, this is not very surprising. Brieger is said to have found neuridine in fish eggs, and Schücking isolated spermine from echinoderm eggs in 1903. Taurine and glycine were found in echinoderm eggs by Kossel & Edlbacher.

Of the manner of formation of ichthulin in the maturation of the ovum we know absolutely nothing. Paton & Newbigin concluded from a very few analyses that the phosphorus was brought to the ovaries from the muscle of the salmon as inorganic phosphorus, but, in view of what is now known about the organic phosphorus compounds of blood, this appears rather unlikely.

1·14. Fats, Lipoids and Sterols

Studies on the fatty substances of the undeveloped eggs of different animals have resulted in much interesting information. There has been, of course, a great body of histological work, and the yolks of all kinds of eggs have been repeatedly subjected to microscopic examination (for example, Kaneko's study on the silkworm); but, in spite of many attempts, I have not succeeded in finding more than a few hints in this literature which are of value to the chemical worker. This subject has been dealt with in a general way by Ransom and by Dubuisson, to whose papers those interested in the histological aspects of yolk must be referred. Of the way in which the fat and the protein are intermingled in the yolk we know practically nothing, and it would be most desirable to investigate the yolk with the methods which modern colloidal chemistry has developed. But that the association between fat and protein indicated by the histological evidence is not very close is shown by the interesting centrifugation experiments of McClendon on the amphibian egg. If the egg of the frog is centrifuged for five minutes under the right conditions, it separates into three perfectly distinct layers, the upper one being oily and yellow, the middle one translucent, colourless and protoplasmic, and the lowest one black, containing practically all the yolk. By using a considerable number of eggs, McClendon was enabled to obtain sufficient material for the chemical analysis of each layer. The figures he obtained are shown in Table 43. It is evident from a slight inspection of his results that the upper layer is composed mainly of neutral fats and a little lecithin, and the middle layer of water, salts and protein, with no fats or lipoids. The lowest and much the largest layer is made up of the vitellin (ranovin or batrachiolin) together with the major part of the lecithin. It is interesting that the association between the phosphoprotein and the lipoid was the only one that centrifuging could not break, for, as we have already seen, the observation of a loose lecitho-vitellin combination in the hen's egg is very old. McClendon found that mitotic figures were all present in the middle layer, and that this centrifuging produced a variety of monstrous embryos. He was led to regard the protoplasm of the egg as constant in composition throughout, but "anisotropic as regards its axes, in other words crystalline in structure".

McClendon extended his observations to the egg of the sea-urchin, *Arbacia punctulata*. Separated by centrifugal force, this egg divided itself into four layers, as Lyon had already described, (*a*) a layer of yolk bodies and red pigment granules extending from the centrifugal end about half-way to the equator, (*b*) a layer of similar yolk bodies but without the pigment granules, (*c*) a translucent fluid layer extending almost to the centripetal pole and containing the nucleus, and finally (*d*) a very opaque layer or cap of minute volume, sitting on the centripetal pole. When the crushed eggs were centrifuged, the material separated into two layers, (*a*) and (*b*) being indistinguishable, centrifugal and containing the egg-membranes, and (*c*) centripetal, (*d*) not being perceptible. McClendon analysed the layers in the same manner as those of the frog's egg—the figures are given in Table 43.

Table 43.

McClendon's figures (1909).

				In the layers		% in the layers dry weight				% of phosphorus					
	Wt. of layers	(% of total weight)		Water %	Solids %	Ether extract	Alcohol extract	Water extract	Final residue	In ether extract	In alcohol extract	In water extract	In final residue	Nitrogen in residue (%)	Ash in residue (%)
Layers of centrifuged egg of *Rana pipiens*	0·06	6	Upper centripetal (fatty or oily)	50	50	80·0	4·0	8	8	Trace	0·34	1·4	0·41	—	—
	0·16	16	Middle (protoplasmic)	82	18	7·5	11·5	60	21	Trace	0·05	1·0	0·37	—	—
	0·78	78	Lower centrifugal (yolky)	48	52	24·0	6·0	10	60	Trace	2·70	1·2	1·33	—	—
Layers of centrifuged egg of *Arbacia punctulata*	—	32·5	Centripetal (protoplasmic)	88	12	49·0	—	20	31·0	2·36		16·66	3·24	13·45	1·24
	—	67·5	Centrifugal (yolky)	79	21	38·2	—	10	51·8	2·74		12·84	1·6	10·6	2·02

A short consideration of them shows that centrifugal force is not nearly so successful in separating the egg of the sea-urchin into chemically unlike layers as it is in the case of the frog. This fits in perhaps with the long-established fact that centrifugal force interferes far less with normal development in the sea-urchin's egg than it does in the frog's egg (Morgan and Lyon). It was very noticeable that, whereas the frog's egg separated out into layers of markedly different water-content, this did not take place in the sea-urchin's egg. In the case of the centrifuged frog's egg, again, there were big differences between the phosphorus contents of the different layers, but in that of the sea-urchin's egg this only applied to the residues which were mainly protein. McClendon surmised that the inclusion of the membrane proteins in the centrifugal layer caused this effect.

It is of course a fact of the first importance that normal development can follow centrifugation and this will receive attention later (see Section 3 and the Epilegomena). I shall only mention here as one of the best instances of this phenomenon, the work of Schaxel on the axolotl egg. Here centrifugation caused atypical discoidal cleavage which nevertheless resulted in a normally proportioned embryo. Thus normal conclusions can follow abnormal distribution of the so-called "organ-forming substances". For further details of these experiments, see Morgan and Bertalanffy.

The early work of Gobley on the fat of the hen's and the carp's egg has already been described. He isolated glycerophosphoric acid from the latter, and pursued further his investigation of lecithin, concerning which it is of interest to note that Sacc contested his claim to have found organic alcohol-soluble phosphorus. Sacc believed that the fats contained dissolved in them a quantity of inorganic phosphorus. Gobley, however, was easily able to disprove this view and to show the identity of carp's egg lecithin with brain lecithin. Data which have accumulated since Gobley's time on the fatty substances of the eggs of the lower animals are collected in Table 44, and may be compared with those in Table 22. One of the most striking differences between the hen's egg and other eggs is the relatively low iodine value of the fatty acids of the former, both free and combined in lipoids. The neutral fat of the hen's egg has an iodine value varying roughly between 60 and 90, but for fish eggs the figures vary from 90 to 150, and the same rule holds generally of the lipoid fatty acids, for they average 60 in hen and 100 in fish eggs. The saponification numbers, on the other hand, are much the same throughout the two tables (from 170 to 200). The conclusion might therefore be drawn that egg fats differ rather more as to the number of unsaturated linkages in their acids, than as to the length of their chains. Nevertheless, there are remarkable exceptions to these generalities, the fatty acids of the echinoderm eggs, for example, having enormous saponification and high Dyer numbers, and therefore presumably only very short chains of carbon atoms. *Arbacia* is more remarkable in this than *Asterias*. Yet, though they are exceptional in that respect, they have iodine numbers very like those of fish-egg fats. Another point of interest is that the cholesterol/fatty acid ratio, as shown by expressing the cholesterol in percentage of the fat present, is rather constant, never going below 4 and never rising above 12. This may have some connection with the physical state of the egg-cell, and will be referred to again (see Section 12·5). The lipoids, expressed as lecithin in per cent. of the fat present, show greater variations, but it is not possible to say at present what the significance of these may be.

Table 44. *Data for fat fraction of eggs.*

Species	Iodine value of neutral fatty acids	Saponification no. of neutral fatty acids	Dyer no. (steam volatile fatty acids) cc. N/10 NaOH required to neutralise distillate	Cholesterol % of the fat fraction	Lecithin % of the fat fraction	Free oleic acid % wet wt.	Free lactic acid % wet wt.	Squalene % of the fat fraction	Unsap. subs. not chol. in % fat fraction	Investigator and date
AMPHIBIA										
Frog (*Rana temporaria*)	123·0	—	—	5·97	—	—	—	—	—	Fauré-Fremiet & Dragoiu (1923)
FISHES										
Sturgeon	107·6	191·4	—	4·35	12·92	—	—	—	—	König & Grossfeld (1913)
Trout	128·3	181·8	—	6·52	41·10	0·19	0·19	—	—	,, ,,
Cod	148·4	176·1	—	12·05	35·19	0·21	0·43	—	—	,, ,,
Herring	123·1	230·6	—	6·94	43·61	—	—	—	—	,, ,,
Carp	78·9	186·9	—	10·98	59·19	—	—	—	—	,, ,,
Pike	—	—	—	—	—	0·27	0·22	—	—	,, ,,
Trout (*Salmo fario*)	108·6	219·8	—	6·23	37·50	—	—	—	1·7	Fauré-Fremiet & Garrault (1922)
	(132·9	189·8	Fatty acids of the phosphatide fraction)							
Carp (*Cyprinus carpio*)	—	140·0	—	5·99	60·00	—	—	—	3·36	,, ,,
	(64·4	—	Fatty acids of the phosphatide fraction)							
Dogfish	(55·88	—	Fatty acids of the phosphatide fraction)							Ponce (1924)
Shark (*Lepidorhinus kinbei*)	—	—	—	—	—	—	—	17·3	—	Tsujimoto (1920)
ECHINODERMS										
Sea-urchin (*Arbacia punctulata*)	147·0	606·0	4·001	—	—	—	—	—	—	Page (1927)
Sea-urchin (*Echinus esculentus*)	145·0	195·0	—	—	29·40	—	—	—	—	Moore, Whitley & Adams (1913)
	(78·8	225·0	Fatty acids of the phosphatide fraction)							
Sea-urchin (*Arbacia punctulata*)	—	—	—	—	50·00	—	—	—	—	Matthews (1913)
Sea-urchin (*Paracentrotus lividus*)	150·0	—	—	—	—	—	—	—	—	Ephrussi & Rapkine (1928)
Starfish (*Asterias glacialis*)	112·5	318·8	3·778	—	—	—	—	—	—	Page (1927)
POLYCHAETE										
Polychaete worm (*Sabellaria alveolata*)	—	—	—	8·85	—	—	—	—	10·6	Fauré-Fremiet (1921)
NEMATODE										
Roundworm (*Ascaris megalocephala*)	—	—	—	5·0	—	—	—	—	80·0	Fauré-Fremiet (1913)

The mention of squalene in Table 44 indicates the existence of an egg-constituent, our knowledge of which is of very recent origin. In 1906 Tsujimoto isolated from the liver oils of elasmobranch fishes a saturated hydrocarbon of approximate formula $C_{30}H_{20}$, and in 1916 published a further study of it. Its properties and constants are given in Table 22. In 1920 he reported that he had been able to isolate it from the egg-yolks of two elasmobranchs, *Chlamydoselachus anguineus* and *Lepidorhinus kinbei*, where it made up no less than 13 per cent. of the egg (wet weight) and, in another case, 17 per cent. at least of the total fat fraction. There the matter rested until 1926, when Heilbron, Kamm & Owens, taking up the question of its presence in eggs once more, isolated it from the undeveloped yolks of *Etmopterus spinax*, *Lepidorhinus squamosus* and *Scymnorhinus lichia*. In the fully developed eggs of the first-named of these three, practically none was present, indicating that it must either have been combusted or absorbed during development. Further researches on the embryological significance of this compound are greatly required. It is possible that some hydrocarbon of this sort may explain certain obscure points in the chemistry of the egg, for instance, the oil extracted by Dubois from the locust's egg (*Acridium peregrinum*). It contained 1·92 per cent. phosphorus, and was present to the extent of 4·5 per cent. of the wet weight of the egg, no small proportion. Kedzie studied a similar oil which he obtained from the egg of the American locust.

A question which is perhaps related to the general problem of the egg-oils is that of the oil-globules of the yolks of some of the teleostean fishes. In 1885 Agassiz & Whitman divided all pelagic eggs into those which had the oil-globule and those which had not. But it was soon found that this method of classification was valueless, for the appearance of the globule is rather erratic; thus, although *Lota vulgaris* (van Bambeke), *Brosmius* (anon.) and *Motella mustela* (Brook) were all found to have it, the common pike's egg does not have it (Truman). Ryder first suggested that the oil-globule might have a relation to buoyancy, but Prince, reviewing the whole subject a little later, pointed out that this could hardly be so, for the salmonoid fishes all have them, and yet their eggs never float. Moreover, out of 22 teleost eggs with no globule, 17 are pelagic, while out of 24 teleost eggs which have globules, only 15 are pelagic. Ryder replied to this by partially withdrawing his theory, and McIntosh simultaneously showed that the eggs of the catfish, which are undoubtedly bottom ova, have large oil-globules. Another theory was put forward by van Bambeke, who believed that the oil-globule was a special form of yolk, and of a purely nutritional significance. Prince criticised this view on the ground that the oil persists in the yolk after the liberation of the embryo from the egg-membrane, and travels beneath it as it swims about. This would not, however, negative the possibility that the oil was used for larval rather than embryonic nourishment. Van Bambeke's claim that a protoplasmic thread passes from the oil-globule to the germinal disc was almost completely disproved by van Beneden. His and Miescher, examining the oil histochemically, found that it only stained very slowly with osmic acid, and therefore differed profoundly from the yolk, and, although it was soluble in ether, it contained no more than a trace of phosphorus. It is remarkable that the oil has never been subjected to a proper chemical examination, especially in view of the extensive zoological literature on it. What we know of its properties faintly hints, perhaps, that it may be a hydrocarbon like squalene, and the whole question, indeed, holds out great possibilities for physiological as well as chemical work. The oil must readily dissolve lipochromes, for the pink pigment of the salmonoids is found in it. Prince's own theory was that the globule was a constituent of ancestral significance, a vestige from the time when, as Balfour showed, the teleostean yolk was very much larger than it is now. The nutrition view is probably the best.

The lipoids and sterols of the eggs of the lower animals are very little known, and their further study is much to be desired. Page in 1923 described a sterol—asteriasterol—which he isolated from the eggs of *Asterias forbesii* and which turned out to be closely related to, though not identical with, ordinary cholesterol; the eggs of *Arenicola cristata*, on the contrary, yielded a sterol absolutely identical with the well-known substance as it occurs in mammals. Ten years previously, in a less accurate study, Matthews had failed to find any cholesterol at all in the eggs of *Asterias forbesii*, though he had been able to isolate some from those of *Arbacia punctata*. From the former he got a jecorin-like substance, containing 10 per cent. of glucosamine, which was probably a mixture of kephalin, cerebrosides, "protagon" and various carbohydrates. Page's later study of the fats and lipoids of the echinoderm egg led to the conclusion that (qualitatively) there was more kephalin in the eggs of *Arbacia* than in those of *Asterias*, and more lecithin in the eggs of *Asterias* than in those of *Arbacia*. *Asterias* contains large amounts of soaps, and its oil is present in much greater abundance than the oil of *Arbacia*; moreover, it contains more sulphur compounds (sulphatides?) decomposable with potash than does the *Arbacia* egg. Page, Chambers & Clowes made a study of the effects of various cytolytic agents on the eggs of *Asterias* separated by microdissection into their cortical and endoplasmic components. They used for this purpose hypotonic sea water, digitonin and saponin, and found that digitonin caused slow cytolysis of the cortical and rapid cytolysis of the interior protoplasm when the two were isolated, whereas hypotonic sea water caused slow cytolysis of the interior and rapid cytolysis of the cortical protoplasm. If these results do not actually demonstrate that the greater part of the asteriasterol is localised in the outer and fertilisable parts of the egg, they at any rate suggest a new method of investigation which may help to solve many similar questions in the future. Runnström has studied the lipoids of the echinoderm egg in relation to its coloured interference fringes and its membrane properties.

Among the sterols existing in eggs must be mentioned a substance which has long been known to occur in the ova of *Ascaris*, and which has been called "ascarylic acid". Fauré-Fremiet identifies it with the droplets or crystals described in the egg of *Ascaris* by van Beneden. It was isolated simultaneously by Fauré-Fremiet from the eggs and by Flury from the whole body of the nematode; the former worker found that it accounted for 22 per cent. of the dry material. Ascarylic alcohol, ascarylic acid, or, as it would probably be best to call it, ascaristerol, seems to exist in the egg-protoplasm in combination with palmitic, oleic, and perhaps stearic acid in ester form. Fauré-Fremiet & Leroux studied its properties, and proposed the provisional formula of $C_{32}H_{64}O_4$. Its saponification number was 199, and its m.p. 82°, it did not give the cholesterol colour-reactions, and its molecular weight was close to 511. Flury considered it to be related to oenocarpol. Acaristerol seems to be strictly confined to the eggs, for even the parietal cells of the ovary and uterus do not contain it, as Fauré-Fremiet showed by means of histochemical tests. Nor is it present in the testes and spermatozoa. It may at present be classed with the sterols, like asteriasterol. *Ascaris* eggs also contain 0·16 per cent. dry weight of ordinary cholesterol.

Table 45. *Distribution of phosphorus.*

Species	In % of the total P							Investigator and date
	Ether-soluble P	Total water-soluble P	Org. water-soluble P*	Inorg. water-soluble P†	Nucleo-protein P	Phospho-protein P	Total protein P	
BIRD								
Hen	61·4	9·5	9·5	None	1·6	27·5	29·1	Plimmer & Scott (1909)
AMPHIBIAN								
Frog (ovarian) ...	26·2	4·3	4·3	None	7·6	61·9	69·5	Plimmer & Kaya (1909)
FISHES								
Sturgeon	28·8	16·9	—	9·9	None	54·3	54·3	Plimmer & Scott (1908)
Herring	—	—	—	—	—	63·0	63·0	,, ,,
Grey mullet ...	—	—	—	—	—	48·0	48·0	,, ,,
Trout	26·0	—	—	—	—	34·6	—	Fauré-Fremiet & Garrault (1922)
Herring	33·2	—	—	—	—	66·8	—	Yoshimura (1913)
Haddock	—	—	—	21·22	—	—	—	Milroy (1898)
Herring	—	—	—	—	10·0	90·0	—	Tschernorutzki (1912)
Salmon	37·8	18·9	—	Traces	—	43·3	—	Paton (1898)
Herring	—	—	—	‡	—	—	—	Steudel & Takahashi (1923)
ECHINODERMS								
Sea-urchin (*Strongylocentrotus purpuratus*)	43·0	33·1	—	—	—	—	23·8	Robertson & Wasteneys (1913)
Sea-urchin (*Arbacia punctulata*)	—	—	—	—	Much	None	—	Masing (1910)
Sea-urchin (*Arbacia punctulata*)	29·2	47·0	—	1·1	—	—	23·5	McClendon (1909)
Sand-dollar (*Dendraster excentricus*)	6·09	46·95	17·55	29·4	32·0	12·1	44·1	Needham & Needham (1930)
Starfish (*Patiria miniata*)	32·3	51·8	32·0	20·0	15·40	Trace	15·4	,, ,,
CRUSTACEA								
Sand-crab (*Emerita analoga*)	28·2	61·40	42·10	19·3	10·82	Trace	10·82	,, ,,
Brine-shrimp (*Artemia salina*)	5·9	56·4	38·3	18·1	37·9	None	37·9	,, ,,
GEPHYREA								
Gephyrean worm (*Urechis caupo*)	27·4	56·90	40·60	16·3	15·80	Trace	15·8	,, ,,
NEMATODE								
Roundworm (*Ascaris*)	25·6	—	—	20·0	54·4	—	54·4	Fauré-Fremiet (1913)

Miescher (1872) reported that in the salmon nearly all the phosphorus is in organic form.

* This fraction will include pyrophosphate P.

† This fraction will include guanidine phosphoric acids (arginine or creatine phosphate P).

‡ "Present in some quantity."

The lipoids of the mammalian egg-cell have recently been the subject of some work which is interesting, though, like all histochemical studies, very difficult to appraise. Following on Russo's claim to have found two different sorts of eggs in rabbits, varying in their reactions to staining methods, Fels in 1926 confirmed this difference for the human egg-cell, some specimens of which showed a strong lipoid-reaction (Ciaccio and Smith-Dietrich methods) in the nucleolus while others did not. Fels' illustration is certainly striking. Leupold had already put forward the view that eggs whose nucleoli were rich in lipoids produced females, and the remainder males, but all the evidence, however, is against sex-dimorphism in the mammalian egg (see Parkes' review). These observations, together with those of Pollak on the presence of Reinke's crystals in the egg of *Macacus rhesus*, and similar work by Limon and von Ebner (on *Cerrus capreolus*), are all that we have on the chemical constitution of the mammalian egg-cell.

Closely connected with the lipoids of the egg is the distribution of phosphorus compounds in it and Table 45 gives what is known upon this subject. It is interesting to see how the phosphoprotein phosphorus varies, in some eggs being very large in proportion to the total phosphorus, in others being almost insignificant. Masing was wrong in saying that the echinoderm egg has none at all, for Needham & Needham in 1929 observed quite a high percentage in the egg of the sand-dollar. It is significant in view of what has already been said about the pre-eminence of birds in storing fat in their eggs, that the hen's egg has 20 per cent. more phosphorus in lipoidal form than any other egg investigated. The fishes rank in this respect with the echinoderms and annelids, little difference being noticeable between alecithic and lecithic eggs. Perhaps this famous distinction involves neutral fat rather than lipoids.

It is to be noted from Table 45 that the inorganic phosphorus content of eggs is very variable; in many cases almost none is present, but the haddock's egg seems to have no less than 20 per cent. of the total phosphorus in this form. About the same proportion is present in the nematode egg, if *Ascaris* can be taken as representative. Fauré-Fremiet was able to identify the calcium phosphate in the egg-cytoplasm with the "hyaline balls" described by van Beneden, using various histochemical reactions (McCallum, Prénant, etc.). Pure calcium phosphate, according to Fauré-Fremiet, accounts for 0·4 to 0·6 per cent. of the dry weight of *Ascaris* eggs, an inconsiderable amount in view of the share it takes in the appearance of the cytoplasm as a whole.

1·15. Carbohydrates

The carbohydrates of the eggs of the lower animals have been less investigated than anything else—a summary of our quantitative knowledge concerning them is shown in Table 46. The presence of glycogen in insect and mollusc eggs was noted by Bernard and in those of arachnids by Balbiani. For the reasons mentioned above, it is difficult to know how trustworthy the figures for carbohydrates are, so bad have the methods been in the past. Fauré-Fremiet & du Streel's figure for the glycogen of the frog's egg must surely be too high, for most of the other workers are agreed on a value of about 2 gm. per cent. wet weight. In the case of animals other than amphibia, the figures are too scattered to permit of any generalisation: thus, though glycogen was not found in herring's eggs by Steudel & Osato, Gori did note its presence in *Torpedo* eggs, and the eggs of the reptiles *Vipera aspis* and *Elaphis quadrilineatus*, in addition to free carbohydrate. Steudel & Osato pointed out that many histologists such as Goldmann had published results concerning the fish egg which might lead one to suppose that very large amounts of glycogen were there. That this was not found by chemical methods ought to induce, they felt, a more cautious attitude towards histochemical work than was customary; indeed, much of what is called glycogen histochemically can certainly not be glycogen. Greene's carbohydrate figures for the eggs of the king-salmon *Oncorhynchus tschawytscha*, from Californian rivers, were of special interest, for, throughout the maturation period, the carbohydrate content of the egg remained the same. The duration of the fast did not affect it at all.

Qualitative investigations of carbohydrate in eggs have been made by Anderlini on the silkworm egg and by Konopacki, who observed the presence of glycogen microchemically in the perivitelline fluid of the frog's egg. As has already been mentioned, a carbohydrate group is undoubtedly contained in the mucoprotein of the amphibian egg-jelly, and von Fürth's analysis of the egg-cases of the squid *Loligo vulgaris* showed that their protein also contained a carbohydrate, but whether these play any part in the sugar supply for the developing embryo remains an obscure point. Haensel found an amount of glucose in the frog's egg which is shown in Table 46, but he also tried the effect of keeping the eggs in solutions of various mono- and di-saccharides,

Table 46.

Species	Mgm. % wet weight			Mgm. % dry weight			Investigator and date
	Total carbohydrate	Free glucose	Glycogen	Total carbohydrate	Free glucose	Glycogen	
REPTILES							
Turtle (*Thalassochelys corticata*)							
White	—	Trace	—	—	—	—	Tomita (1929)
Yolk	—	100	—	—	—	—	,,
Tortoise (*Testudo graeca*) ...	—	140	—	—	—	—	Diamare (1910)
AMPHIBIA							
Frog (*Rana esc.* and *fusc.*) ...	—	—	2,520	—	—	—	Kato (1909)
,, ,, ...	—	—	1,100	—	—	—	Athanasiu (1899)
Frog (*Rana temp.*)	—	—	—	—	—	7810	Fauré-Fremiet & Dragoiu (1923)
Frog (*Rana esc.* and *fusc.*) ...	—	—	2,500	—	—	—	Bleibtreu (1910)
Frog (*Rana temp.*)	—	—	—	—	193	—	Gori (1920)
,, ,, ...	—	—	10,140	—	—	—	Fauré-Fremiet & Vivier du Streel (1921)
,, ,, ...	604	—	—	1906	—	—	Needham (1927)
,, ,, ...	—	—	2,528	—	—	—	Haensel (1908)
,, ,, ...	—	—	1,650	—	—	—	Goldfederova (1925)
FISHES							
Herring	—	500	—	—	—	—	Steudel & Osato (1923)
King-salmon (*Oncorhynchus tschawytscha*)	—	96	—	—	—	—	Greene (1921)
Trout (*Salmo fario*)	—	—	340	—	—	—	Fauré-Fremiet & Garrault (1922)
ECHINODERMS							
Starfish (*Asterias glacialis*) ...	—	—	20	—	—	—	Dalcq (1923)
Sea-urchin (*Echinus esculentus*)	—	—	1,360	—	—	8980	Moore, Whitley & Adams (1913)
Sea-urchin (*Strongylocentrotus lividus*)	1360	—	—	5430	—	—	Ephrussi & Rapkine (1928)
INSECTS							
Silkworm (*Bombyx mori*) ...	—	—	1,110	—	—	—	Pigorini (1922)
,, ,, ...	—	—	1,980	—	—	—	Tichomirov (1882)
,, ,, ...	—	—	—	—	—	3080	Vaney & Conte (1911)
Bee (*Apis mellifica*)	—	—	2,500	—	—	—	Straus (1910)
CEPHALOPOD							
Octopus (*Sepia officinalis*) ...	—	—	None	3620	1000	None	Henze (1908)
,, As glucose	2700	—	—	—	—	—	,,
,, As pentose	2380	—	—	—	—	—	,,
POLYCHAETE							
Polychaete worm (*Sabellaria alveolata*)	—	—	1,270	—	—	—	Fauré-Fremiet (1921)
NEMATODE							
Roundworm (*Ascaris megalocephala*)	—	—	—	—	—	2105	Fauré-Fremiet (1913)

Table 47. *Ash content of eggs.*

Species	Water %	Ash gm. % wet wt.		% of total ash								Mgm. per 100 gm. wet wt. (or per 100 c.c. sea water)									Millimols present								Milliequivalents present								Total cation		Total anion		Investigator and date	Anion/cation ratio in milli-equiv.
				Cation					Anion																																	
		Direct	By diff.	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	Si	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	K	Na	Mg	Ca	Fe	SO_4	PO_4	Cl	Milli-mols	Milli-equiv.	Milli-mols	Milli-equiv.		
WHOLE EGG OR ITS PARTS																																										
Pike (*Esox lucius*) ...	66·53	2·06	2·87	11·11	7·14	2·01	4·47	0·03	26·01	47·45	1·78	229	147	41·4	92	6	536	978	37	—	5·87	6·39	1·73	2·3	0·107	5·58	10·03	1·04	5·87	6·39	3·46	4·6	0·214	11·16	30·09	1·04	16·29	*20·32*	16·65	*42·29*	Konig & Grossfeld (1913)	*2·08*
Cod (*Gadus morrhua*) ...	73·04	1·68	3·08	6·80	6·62	1·08	2·69	0·07	26·44	47·83	8·46	114	111	18	45	1·2	444	804	142	—	2·92	4·83	0·75	1·1	0·0214	4·03	8·46	3·98	2·92	4·83	1·50	2·2	0·0652	9·26	25·38	3·98	9·62	*11·51*	17·07	*38·62*	,, ,,	*3·35*
Sturgeon (*Acipenser sturio*)	—	4·55	6·38	5·02	—	3·76	2·85	0·71	43·64	44·2	—	228	—	171	130	32	1990	2010	—	—	5·84	—	7·13	3·25	0·57	20·70	21·15	—	5·84		14·26	6·50	1·71	41·4	63·45	—	16·79	*28·31*	41·85	*104·85*	,, ,,	*3·70*
Sea-urchin (*Arbacia*) ...	80·0(?)	2·00	—	21·7	11·55	39·8	16·9	—	0·0041	5·69	1·66	434	231	796	338	0·48	0·082	114	33·2	—	1·015	0·903	2·93	0·758	0·0081	0·0006	0·469	0·085	1·015	0·903	5·86	1·52	0·024	0·0012	1·41	0·085	5·61	*9·32*	0·555	*1·496*	Page (1927)	*0·16*
Starfish (*Asterias*) ...	80·0(?)	2·00	—	38·6	34·5	12·0	8·48	—	—	19·4	—	772	690	240	169	—	—	388	—	—	19·80	30·0	10·0	4·22	—	—	4·09	—	19·8	30·0	20·0	8·44	—	—	12·27		64·0	*78·24*	4·09	*12·27*	,,	*0·15*
Sea water (Woods Hole)	—	3·19		1·3	27·6	4·1	1·3		8·2	0·006	57·5	41	880	130	43	—	261·5	0·2	1835	—	1·05	38·3	5·4	1·07	—	2·72	0·002	51·7	1·05	38·3	10·8	2·14	—	5·44	0·004	51·7	45·82	*52·29*	54·42	*57·14*	,,	*1·09*
Sea-urchin (*Arbacia*) ...	—	—	—	—	—	—		—	—	—	—	—	—	—	—	0·02	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Warburg (1914)	—
Sea water (*Challenger* expedition)	—	3·48	—	1·105	30·8	3·73	1·2	—	6·41	—	55·3	38·4	1070	130	41·8	—	223	—	1925	—	0·99	46·6	5·4	1·04	—	2·32	—	54·2	0·99	46·6	10·8	2·08	—	4·64	—	54·2	54·03	*60·47*	56·52	*58·84*	Dittmar, *Challenger*, vol. 1	*0·97*
Carp ...	64·08	0·853		34·2	0·94	—	13·2	—	0·92	22·3	28·5	292	8	—	113	—	7	190	243·4	—	7·49	0·35	—	2·8		0·31	2·0	6·9	7·49	0·35	—	5·6	—	0·62	6·0	6·9	10·64	*13·44*	9·21	*13·52*	Gobley (1850)	*1·00*
Sea-urchin (*Strongylocentrotus lividus*) ...	77·9	2·14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	310·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Wetzel (1907)	—
Dogfish (*Scyllium canicula*) ...	48·6	2·83	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	925	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,	—
Spider-crab (*Maia verrucosa*) ...	56·4	1·79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	877	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,	—
Octopus (*Sepia officinalis*) ...	52·7	1·04	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	728	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,	—
Grass-snake (*Tropidonotus natrix*) ...	43·3	3·80	—			—		—	—	—	—	—	—	—	—	—	—	1370	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				—	—	Sommer & Wetzel (1904)	—
Hen (*Gallus domesticus*)...		1·72		10·0	1·2	1·2	8·1	—	—	64·2	15·2	175	21	20	140	—	—	1107	262	—	4·5	0·91	0·83	3·5		—	11·65	7·38	4·5	0·91	1·66	7·0	—	—	23·3	7·38	9·79	*14·11*	19·03	*30·68*	Bialascewicz (1926)	*2·17*
Mollusc (*Voluta brasiliense*) ...	74·52	4·08		—	33·7	—	1·35	—	6·44	—	51·9	—	1340	—	54	—	260	—	2070	—	—	58·3	—	1·35	—	2·71	—	58·3	—	58·3	—	2·70	—	5·42	—	58·3	59·65	*61·0*	61·01	*63·72*	Roffo & Correa (1927)	*1·04*
Dogfish (*Squalus acanthias*) ...	52·68	2·03	—	6·4	4·3	—	—	—	42·3	43·9	3·3	130	87	—	—	—	858	891	68	—	3·33	3·63			-	8·94	9·38	1·9	3·33	3·63	—	—	—	17·88	28·14	1·9	6·96	*6·96*	20·22	*47·92*	Zdarek (1904)	*6·89*
Frog (*Rana temporaria*) ..	—	2·22		10·2	1·9	2·9	0·85	—	—	77·0	7·1	227	42	65	19	—	—	1713	158	—	5·82	1·75	2·71	0·47	—	—	18·0	4·4	5·82	1·75	5·42	0·94	—	—	54·0	4·4	10·75	*13·93*	22·4	*58·4*	Bialascewicz (1926)	*4·19*
Trout (*Salmo fontinalis*)	—	1·51	—	14·4	1·6	3·9	3·1	—	—	66·2	10·8	218	25	60	47	—	—	999	163	—	5·59	1·04	2·5	1·18	—	—	10·5	4·6	5·59	1·04	5·0	2·36	—	—	31·5	4·6	10·31	*13·99*	15·1	*36·1*	,,	*2·58*
Salmon (*Salmo salar*) ...		1·57		15·2	1·1	2·9	6·6	—	—	66·5	7·6	239	18	45	103	—	—	1044	119	—	6·13	0·75	1·87	2·58		—	11·0	3·3	6·13	0·75	3·74	5·16	—	—	33·0	3·3	11·33	*15·78*	14·3	*36·3*	,,	*2·30*
Wrasse (*Labrax lupus*) ...	—	0·76	—	38·8	0·6	1·05	2·6	—	—	41·4	15·3	295	5	8	20	—	—	315	116	—	7·56	0·21	0·33	0·5	—	—	3·3	3·3	7·56	0·21	0·66	1·0	—	—	9·9	3·3	8·60	*9·43*	6·6	*13·2*	,,	*1·40*
Torpedo (*Torpedo ocellata*) ...	-	1·76		11·7	—	0·4	1·7	—	—	77·9	8·2	206	—	7	30	—	—	1371	144	—	5·28	—	0·29	0·75	—	—	14·4	4·1	5·28	—	0·58	1·5	—	—	43·2	4·1	6·32	*7·36*	18·5	*47·3*	,,	*6·43*
Dogfish (*Scyllium canicula*) ..	—	1·765		12·4	4·65	0·6	1·5		—	66·3	14·6	219	82	11	26	—	—	1170	257		5·61	3·42	0·46	0·65	—	—	12·3	7·24	5·61	3·42	0·92	1·3	—	—	36·9	7·24	10·14	*11·25*	19·54	*44·14*	,,	*5·92*
Spider-crab (*Maia verrucosa*) ...	—	1·80	—	8·8	3·0	0·8	2·0			81·0	4·7	158	54	15	36	-	-	1458	84	—	5·1	2·24	0·62	0·9	—	-	15·3	2·4	5·1	2·24	1·25	1·8	—	-	45·9	2·4	8·86	*10·39*	17·7	*48·3*	,,	*4·65*
Octopus (*Sepia officinalis*) ...		1·234		2·7	0·7	0·6	0·9	—	—	81·9	13·9	34	9	7	12	—	—	1011	171	—	0·87	0·37	0·29	0·3		—	10·65	4·8	0·87	0·37	0·58	0·6			31·95	4·8	0·83	*2·42*	15·45	*36·75*	,,	*15·20*
Sea-urchin (*Arbacia pustulosa*)		1·39	—	32·8	3·02	1·73	2·1		—	60·5		456	42	24	29	—		840	—		11·7	1·75	1·0	0·72	—		8·84	—	11·7	1·75	2·0	1·45	—	—	26·52		0·17	*16·90*	8·84	*26·52*	,,	*1·57*
Sea-urchin (*Paracentrotus lividus*)		1·33		40·3	1·85	3·3	3·0	—	—	51·4	—	536	24	44	40	—		684	—	—	13·8	1·0	1·84	1·0		—	7·2	—	13·8	1·0	3·68	2·0		—	21·6	—	7·04	*20·48*	7·2	*21·6*	,,	*1·05*
Gephyrean worm (*Sipunculus nudus*)		0·54		26·9	6·7	2·2	3·3	—	—	61·7	—	145	36	12	18	—	—	333	—	—	3·72	1·5	0·5	0·45	—	—	3·5	—	3·72	1·5	1·0	0·9	—	—	10·5	—	6·17	*7·12*	3·5	*10·5*	,,	*1·74*
Lugworm (*Arenicola claparedii*)	-	0·99		35·9	2·9	9·1	4·3	-	—	47·6	—	355	29	90	43	—		471	—	—	9·1	1·21	3·75	1·08	-	—	4·96		9·1	1·21	7·5	2·16	—	—	14·88	-	5·14	*19·97*	4·96	*14·88*	,,	*0·74*
Herring (*Clupea harengus*) Total ...	-	0·579	—	30·9	14·3	2·6	1·6		—	—	50·8	179	82·5	15	9	—	None	None	294	—	4·59	3·59	0·625	0·225	-	—	—	8·28	4·59	3·59	1·25	0·45	—	—	—	8·28	9·03	*9·88*	8·28	*8·28*	McCallum (1926)	*0·84*
Water-sol.		—				—	—	—	-	—	—	179·5	81·7	1·38	4·6	—	None	None	294	—	—	—	—	—	—	—	—	—	—	—	—	—	—		-	—	—	—	—		,,	—
Salmon ...	—	—	—	—		—	—	—	-	—	—	—	—	—	—	2·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	Greig (1898)	—
Plaice ...	91·66		-	—	—	—	—	-	-	—	—	—	232	—	—	—	—	420	358	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—		Milroy (1898)	—
Dogfish (*Squalus acanthias*) (egg-jelly)	88·75	1·87		29·0		4·0	3·0	—	5·0	14·0	44·0	542		7·5	5·6	—	9·3	261·5	823		14·6		0·31	0·14	—	0·097	2·75	23·2	—	14·6	0·62	0·28	-	0·194	8·25	23·2	15·05	*15·5*	26·05	*31·64*	Perugia (1879)	*2·04*
Sawfish (*Pristis antiquorum*) ...	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	30	460	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Krukenberg (1888)	—
Torpedo (*Torpedo ocellata*) ...	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	50	210	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—			,,	—
Dogfish (*Squalus acanthias*) (whole) ...		—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	96	310	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—		—		,,	—
Silkworm (*Bombyx mori*) ...			—	29·5	1·1	10·3	6·4	-	—	53·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	-	—		—		Peligot (1852)	—
Locust (*Schistocerca paranensis*) ...			—	—		—	—	—	—	—	—	*400*	—	*370*	*30*	*210*	—	*210*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				-	Schröder (1909)	—
Turtle (*Thalassochelys corticata*)																																										
Whole egg ...	—	—	-	14·2	17·2	2·5	6·4	-	15·5	22·2	20·1	97	118	17	44	—	267	348	140	11	2·5	5·1	0·71	1·0	-	8·3	11·2	3·9	2·5	5·1	1·42	2·0	—	16·6	33·6	3·9	—	*11·02*	—	*54·1*	Karashima (1929)	*4·9*
White ...	—	—		14·4	31·2	0·5	1·0	—	2·5	1·0	49·5	34	74	1	2	—	15	5	116	—	0·87	3·2	—	—	—	0·5	—	3·3	0·87	3·2	—	—	—	1·0	—	3·3		*4·07*		*4·5*	,,	*1·06*
Yolk ...	—	—	—	14·0	10·2	3·5	9·3	-	22·2	33·5	5·3	63	46	16	42	—	252	343	24	11	1·6	2·0	0·66	1·0	—	7·9	11·2	0·8	1·6	2·0	1·31	2·0	—	15·8	33·6	0·8	—	*6·91*	—	*50·2*	,,	*7·3*
INTERMICELLAR LIQUID OF THE YOLK																																										
Hen (*Gallus domesticus*) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10·1	2·8	1·8	2·7	—	—	1·9	15·4		*17·4*		*17·3*	Bialascewicz (1928)	—
Frog (*Rana temporaria*) ...	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10·8	1·9	4·5	0·7	—	—	15·9	7·4		*17·9*		*22·3*	,,	—
Trout (*Salmo fontinalis*) ...		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8·2	1·9	2·6	0·9	—	—	2·3	8·1		*13·6*		*10·4*	,,	
Torpedo (*Torpedo ocellata*) ...				—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14·3	0·7	0·2	1·3	—	—	—	20·6	—	*16·5*		*20·6*	,,	
Spider-crab (*Maia verrucosa*) ...					—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11·3	0·3	2·8	2·5	—	—	4·2	11·5	—	*16·9*		*15·7*	,,	—
Sea-urchin (*Paracentrotus lividus*) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24·2	1·1	1·6	1·5	—	—	4·6	38·7		*28·4*		*43·3*	,,	—
Octopus (*Sepia officinalis*) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·2	0·5	0·8	1·6	—	—	—	10·1	—	*4·1*		*10·1*	,,	—

to see if they would grow richer in glycogen. They all did; in fact, he was able to double their glycogen content by this simple means (glucose acted better than sucrose, sucrose than lactose, and lactose than glycerol, though even the latter substance gave an effect). These curious observations have never been confirmed, and can hardly be said to carry conviction as they stand. Diamare obtained discordant results in his researches on the sugar of various eggs; thus, he got a rather low value for the free glucose of the egg of *Testudo graeca*, but none at all, either free or combined, from the eggs of *Scyllium catulus* or *Torpedo marmorata*. No explanation can be given for this fact. In connection with carbohydrates, it should be remembered that viper venom, which is in all probability a glucoside, has been shown by Phisalix to be present in active form in the yolks of viper eggs.

1·16. Ash

We come now to the inorganic substances of eggs. Iron has been shown to be present by microchemical tests in many eggs, such as those of *Limnaea*, *Tubifex*, *Rana esculenta* (where it is massed at the light ventral pole) and *Pisidium*, by the work of Schneider. Dhéré found traces of iron and copper in the eggs of *Sepia*. Warburg found 0·02 to 0·03 mgm. iron per 100 mg. nitrogen in the unfertilised sea-urchin egg; part of it seemed to be in ionic form and part not. According to Wilke-Dörfurt, there are 4·8 mgm. per kilo iodine in oyster egg-shells.

Ash analyses of eggs have been made by several workers, whose results, it may be remarked, would have been more easily comparable if they had expressed them in the same way, instead of in nine or ten different ways, omitting in some cases the figures which would enable them to be calculated into a form comparable with each other. Table 47 summarises what is known about the distribution of inorganic substances in eggs. It has entailed a good deal of calculation, for only one of the previous investigators expressed his results in terms of millimols and milliequivalents, and unless this is done it is impossible to gain any idea as to the relative preponderance of cation and anion. The first thing which should be noted is the fact that, when the salts are expressed in per cent. of the total ash, potassium is always there in greater amount than sodium, and nearly always to a greater extent than any other metal. This seems to be quite characteristic of the ovum, though in other systems of

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1·16. Ash

We come now to the inorganic substances of eggs. Iron has been shown to be present by microchemical tests in many eggs, such as those of *Limnaea*, *Tubifex*, *Rana esculenta* (where it is massed at the light ventral pole) and *Pisidium*, by the work of Schneider. Dhéré found traces of iron and copper in the eggs of *Sepia*. Warburg found 0·02 to 0·03 mgm. iron per 100 mg. nitrogen in the unfertilised sea-urchin egg; part of it seemed to be in ionic form and part not. According to Wilke-Dörfurt, there are 4·8 mgm. per kilo iodine in oyster egg-shells.

Ash analyses of eggs have been made by several workers, whose results, it may be remarked, would have been more easily comparable if they had expressed them in the same way, instead of in nine or ten different ways, omitting in some cases the figures which would enable them to be calculated into a form comparable with each other. Table 47 summarises what is known about the distribution of inorganic substances in eggs. It has entailed a good deal of calculation, for only one of the previous investigators expressed his results in terms of millimols and milliequivalents, and unless this is done it is impossible to gain any idea as to the relative preponderance of cation and anion. The first thing which should be noted is the fact that, when the salts are expressed in per cent. of the total ash, potassium is always there in greater amount than sodium, and nearly always to a greater extent than any other metal. This seems to be quite characteristic of the ovum, though in other systems of the organism other relations are found; thus corpuscles and plasma of some mammalian bloods have converse potassium/sodium ratios, and, as a general rule, potassium preponderates in cells while sodium preponderates in media. Of the anions PO_4 usually takes up much the greatest part, but SO_4 may in certain cases equal it. In the columns on the right of the table the total anion and total cation are shown, in each case calculated as millimols and as milliequivalents, the former giving an idea of the total number of molecules present, the latter of the total number of valencies. Study of the anion/cation ratio expressed as milliequivalents per cent. wet weight provides an important key to the constitution of the egg, for it shows roughly to what extent anion or cation is held in combination with protein or lipoid, or other organic substances. We have already seen that in the case of the hen's egg, taking both yolk and white into account, the anion/cation ratio is more than unity (Bialascewicz's figures give 2·17), showing that a quantity of sulphur and phosphorus is in organic combination—a conclusion which fits in admirably with all that we know of the hen's egg from other sources. The same relationship is seen in the figures of König & Grossfeld for the three fish eggs they investigated, the pike, the cod and the sturgeon. On the other hand, the figures of Page for two echinoderm eggs give ratios much less than unity, demonstrating the organic combination of a good deal of the cation. It may be noticed that the analyses of Dittmar and Page for sea water give ratios in the very close neighbourhood of unity, as would be expected, and indicate at the same time that the ratio cannot be regarded as significant to less than 0·09. From what has been said, therefore, it might be concluded that the yolk-laden eggs of the fishes, like that of the hen, have a ratio above unity, while the alecithic echinoderm eggs have ratios much below it. But there are exceptions to this generalisation. The ratio of unity for the carp egg which is given by Gobley's results may perhaps be neglected, owing to the date of the work (1850), and the similar value obtained by Roffo & Correa on a gastropod egg may also be regarded as suspicious because of the enormous amount of sodium chloride that appears in their analysis. But the careful work of Bialascewicz in 1926 does not altogether support the generalisation. His figures for the fish egg are in good agreement with those of König and Grossfeld, but his anion/cation ratios for the echinoderms do not go below unity, though they approach it much more nearly than do the fishes. Further work is needed to clear up this contradiction. In one case, however, Bialascewicz got a ratio below unity, that of *Arenicola claparedii*, so that in a general sense his investigations are not opposed to those of Page and König & Grossfeld. McCallum's low ratio for the egg of the herring is difficult to explain, but Perugia's analysis of the egg-jelly of ovoviviparous selachians fits in well enough with the majority of the other evidence. Attention might also be drawn to Bialascewicz's high ratio (15) for the eggs of the octopus, *Sepia*, which would appear to be extraordinarily poor in metallic ions (cf. p. 317, Section 13 and the Epilegomena).

Some further light is perhaps thrown on the inorganic composition of eggs by Wetzel's figures for insoluble and soluble ash. He submitted the eggs of various animals to examination, with the following results:

	% dry weight		
Species	Total ash	Insol. ash	Sol. ash
Sea-urchin (*Strongylocentrotus lividus*) ...	9·7	2·4	7·2
Spider-crab (*Maia squinado*)	4·12	0·27	3·8
Octopus (*Sepia officinalis*)	2·2	0·59	1·6
Dogfish (*Scyllium canicula*)	5·5	1·15	4·3

In all cases he found more soluble than insoluble salts, i.e., more chlorides than sulphates and phosphates.

Table 48. *Bialascewicz's figures.*

Species	c.c. of yolk taken	Vol. of liquid after dilution (c.c.)	Degree of dilution	Concentration Cl in 1 c.c. ultra-filtrate (mg.)	Total Cl in ultra-filtrate (mg.)	Vol. of inter-micellar fluid per 1 c.c. yolk
Hen (yolk)	4·8	10	2·08	1·08	1·080	—
,,	4·8	20	4·17	0·472	0·944	0·541
,,	4·8	30	6·25	0·306	0·918	0·569
,,	4·8	40	8·33	0·222	0·888	0·537
,,	4·8	50	10·40	0·195	0·975	(0·754)

It is very interesting, as Bialascewicz points out, that the mineral composition of terrestrial and aquatic animals should be so alike. The preponderance of potassium which is seen in the hen's egg does not change as one passes to organisms laying their eggs in an environment containing far more sodium than potassium. Thus, although the normal sea water has twenty times as much sodium as potassium, fish eggs often have quite twenty times as much potassium as sodium. There would not appear to be in this connection any difference between homoio-osmotic and poikilo-osmotic aquatic animals. It is also obvious from Table 47 that aquatic eggs often have very much less salt in them than the ambient medium, and this would be a special case of the phenomenon found in all marine animals, and termed by Fredericq "Mineral hypotonicity". Bialascewicz arranged the animals he studied in a list of ascending concentration of metallic ions as follows:

Species	Metal gramions per litre
Octopus (*Sepia officinalis*)	0·016
Gephyrean worm (*Sipunculus nudus*) ...	0·064
Spider-crab (*Maia verrucosa*)	0·079
Wrasse (*Labrax lupus*)	0·091
Herring (*Clupea harengus*) (McCallum) ...	0·100
Dogfish (*Scyllium canicula*)	0·107
Sea-urchin (*Arbacia pustulosa*)	0·159
Sea-urchin (*Paracentrotus lividus*)	0·180

which would also be an ascending table of taxonomic groups, were it not for the high metal content of the echinoderm eggs, which exceed even the fishes.

There are other points concerning the relative amounts of salts in the eggs which require mention. McCallum, who had for a long time previously been studying the proportion of salts in the ash of animals and parts of animals with reference to the composition of sea water both now and in earlier geological epochs, made an analysis of herring's eggs in 1926. He had previously differentiated between palaeo-chemical salt ratios in bloods, namely, ratios resembling that which pre-Cambrian sea water can be calculated to have possessed, and neo-chemical salt ratios, namely, ratios resembling the sea water of the present day. Thus *Limulus polyphemus* and *Aurelia flavidula*, the king-crab and the medusa, which have always been marine animals, now approach the modern sea in the composition of their vascular fluids, but the lobster *Homarus americanus*, the selachian *Acanthias vulgaris*, the frog, dog, and man, for instance, all have ratios resembling the composition of the sea water at the appearance of the protovertebrate form. He had also identified the kidney as the organ responsible for maintaining the palaeo-ratios in the salts of the blood. In order to explore the possibility of identifying a palaeo-ratio in the contents of the cell itself, he had recourse to eggs, and for those of the herring obtained the following distribution:

Ratios on the basis of Na 100

Na	K	Ca	Mg	Cl
100	216·7	11·4	18·7	356·8

This stood in marked contrast not only with the vertebrate blood-plasma but also with the Archaean sea water calculated for the time at which life first began to appear in it, thus:

Vertebrate blood-plasma (dog)

100	6·6	2.8	0·7	139·5

Archaean sea water

100	100–250	10	0·05	

But after extraction of the dried eggs with water in a Soxhlet apparatus, the determination of the ratio of salts in the soluble part gave results more like the ratio for the Archaean sea water:

100	219·9	5·6	1·6	359·2

McCallum therefore concluded that the soluble part of the ash of the herring's egg exhibits a palaeo-chemical ratio. The bond shown here between the metals and the organic substances is useful in reminding us that even in fish eggs, where the anion/cation ratio is well above unity, some of the metal as well as the acid radicles may be united in organic combination.

The relation between the salts in the intermicellar fluid of yolk and those in the dispersed phase itself has been studied by Bialascewicz and by Vladimirov. Bialascewicz worked firstly with the yolks of *Torpedo* eggs, but also with those of the hen and the trout. He prepared series of mixtures of the yolk with diluents in different concentrations, such as isotonic solutions of lithium sulphate and lithium nitrate, or in some cases distilled water, and then, submitting the mixtures to ultra-filtration, he estimated the ash and its composition in the filtrate and the residue. He first found that the percentage of chlorine bound to the dispersed phase in the ooplasm was practically independent of the degree of dilution, and from this fact he was able to calculate the volume of the intermicellar fluid of the yolk (see Table 48). For the hen's egg this was 0·549 c.cm. per c.cm. wet substance, and for the egg of *Torpedo ocellata* a similar calculation, based on cryoscopic experiments, gave a value of 0·482. On the basis of these figures, he proceeded to study the partition coefficient of each individual ion as between dispersed phase and intermicellar liquid. In Table 49 these partition coefficients are given; they represent the ratio *amount of ion in the continuous phase or intermicellar liquid/amount of ion in the dispersed phase*. It will be noted from Fig. 17 that as dilution of the original yolk goes on the ratios in some cases change, but in others remain constant. Thus the chlorine of the trout and the hen egg yolk remains constant at 0·5 in the latter and 1·02 in the former case, showing that it is very stably combined in the dispersed phase, though in different proportions according to the animal. Thus there is considerably more chlorine in the dispersed than in the continuous phase of the yolk of the avian egg, while in the fish egg there is a very slight excess of chlorine in the continuous phase. In all other instances, however, both as regards the hen and the trout, the excess of ion is in favour of the dispersed phase, the colloidal aggregates of which may therefore be looked upon as reservoirs of ash. Nevertheless, there is a good deal of the sodium combined in the continuous phase, and not a little of the potassium, though here the trout differs from the hen, for the potassium ratio is about 0·9 in the former case and only 0·7 in the latter. All the other ions have lower ratios than these; magnesium, calcium and phosphorus, for instance, are all present to a much greater extent in the dispersed than in the continuous phase. These experiments show also exactly how firmly the ions in the dispersed phase are bound there, and with what ease they may be washed out into the ultra-filtrate. It is apparent from

Table 49. *Bialascewicz's figures.*

	Egg									
Ion	*Gallus domesticus*	*Rana temporaria*	*Salmo fontinalis*	*Labrax lupus*	*Torpedo ocellata*	*Scyllium canicula*	*Maia verrucosa*	*Arbacia pustulosa*	*Paracentrotus lividus*	*Sepia officinalis*
K	0·722	1·000	0·890	0·768	1·000	0·870	0·967	1·000	0·945	0·800
Na	0·942	0·567	0·509	0·331	0·051	—	0·080	0·728	1·000	1·000
Ca	0·093	0·391	0·274	0·169	0·321	0·760	0·474	0·696	0·505	1·000
Mg	0·295	0·460	0·321	0·380	0·157	0·410	0·707	0·631	0·272	0·491
P	0·025	0·244	0·100	0·275	—	—	0·040	0·318	0·186	—
Cl	0·555	0·905	1·000	0·567	0·943	1·000	0·970	1·000	1·000	0·766

Fig. 17 that the phosphorus, chlorine, and probably sodium in the dispersed phase, are intimately united there, for, however great the dilution of it, they do not increase in the ultra-filtrate. Magnesium and calcium, on the other hand, show a comparative readiness to pass out of the dispersed phase as the dilution is increased. The behaviour of the potassium is the most peculiar, for, as dilution goes on, the calculated concentration of this ion actually decreases, but as the decrease is slight it is probably due to experimental error, and it was treated as such by Bialascewicz himself. Thus, of the ions bound to the dispersed phase, the cations sodium and potassium, and the anions of chlorine and (presumably) phosphate, are firmly attached, while the cations calcium and magnesium are not, and can easily be washed out. The high proportion of phosphorus in organic combination should be remembered here.

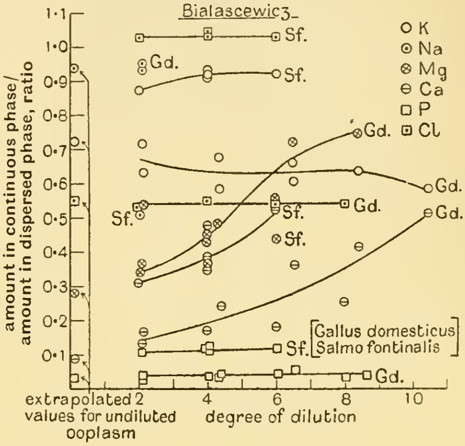

Fig. 17.

Bialascewicz also pointed out that the partition coefficient or ratio followed with dilution a practically rectilinear course, so that some idea of the ratio in the natural undiluted yolk might be obtained by extrapolation. These figures so obtained are shown in Fig. 17, from which it may be deduced that the ions follow the order phosphorus, calcium, magnesium, chlorine, potassium, sodium, beginning with the one most of which is in the dispersed phase and ending with the one least of which is so distributed.

Fig. 18 shows another aspect of the passage of ash from dispersed to continuous phase.

In succeeding papers Bialascewicz extended these researches to the eggs of amphibia, some other fishes, crustacea, molluscs, echinoderms and annelids. He reported that the intermicellar liquid varied much in its relative amount, accounting for from 20 to 63 per cent. of the whole ooplasm. From the data in Table 50, however, there does not seem to be a very close relation between the relative volume of continuous phase and the percentage dry weight of the system. Bialascewicz's tables give the concentration in percentages of the principal ions in the intermicellar liquid of different eggs, and these are conveniently summarised in Fig. 19, taken from his paper. From this it is obvious that all the eggs studied have about the same proportion of potassium, but that the other ions are rather variable. There is much more calcium, relatively, in the continuous phase of the yolk of the hen's egg than in that of any of the others except the crustacean *Maia verrucosa*. Similarly, there is more magnesium, relatively, in that of the frog than in any other egg. A very interesting comparison may be made between the distribution of ions in the continuous phase of the eggs and that in the serum of

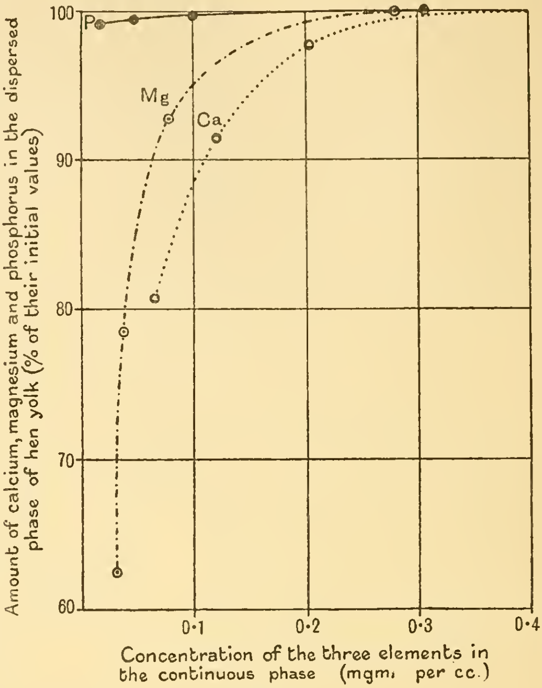

Fig. 18.

Table 50. *Bialascewicz's figures.*

Egg	Continuous phase		% dry weight
	c.c. per c.c. ooplasm	In % of vol. ooplasm	
Scyllium canicula ...	0·83	17·0	—
Salmo fontinalis ...	0·79	20·8	—
Salmo fario	—	—	41·5 (Fauré-Fremiet & Garrault)
Torpedo ocellata ...	0·41	59·0	—
Acanthias vulgaris ...	—	—	47·3 (Zdarek)
Arbacia pustulosa ...	0·82	17·8	—
Paracentrotus lividus ...	0·79	20·7	22·6 (Wetzel)
Rana temporaria ...	0·60	39·9	42·6 (Kolb, Terroine, etc.)
Gallus domesticus ...	0·55	45·1	50·3 (Kojo)
Sepia officinalis ...	0·50	50·0	47·3 (Wetzel)
Maia verrucosa ...	0·37	63·2	43·6 (Wetzel)

the corresponding adult animals. Fig. 20, taken from Bialascewicz, shows that the potassium preponderates in the former and the sodium in the latter, while the other inorganic substances are more or less equally distributed. As there is no difference in electrolyte concentration between the continuous ooplasm phases of fresh-water and marine animals, one must conclude that salts do not account for the properties possessed by the latter, and that crystalloidal organic compounds, such as taurine, urea and glycine, play an important part in keeping up a high osmotic pressure. Thus Bialascewicz found a concentration of 8·43 gm. per litre of urea in

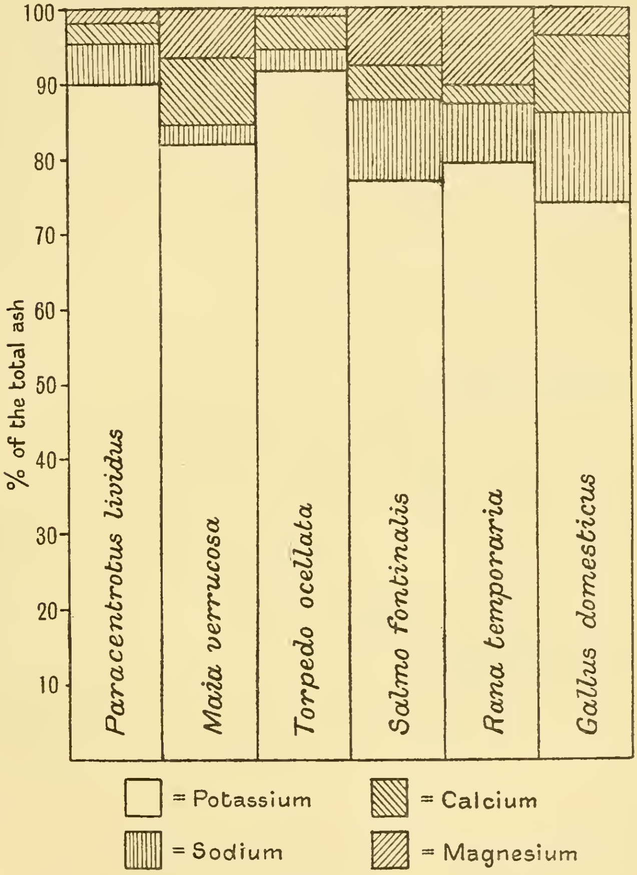

Fig. 19.

undeveloped *Torpedo ocellata* eggs, but none in those of *Arbacia*, *Sepia*, or *Maia*.

Vladimirov occupied himself with the egg-white in the egg of the bird. In connection with other investigations which dealt with the water metabolism of the egg (see Section 6·4) he measured the electrical conductivity of the egg-white in the unfertilised ovum, using the Kohlrausch and Holborn apparatus, and obtaining a value of $7 \cdot 6 \times 10^{-3}$. By the aid of a dialysis method he calculated the electrical conductivity of the intermicellar fluid of the egg-white, allowing for the disturbing effect of so large a concentration of pro-

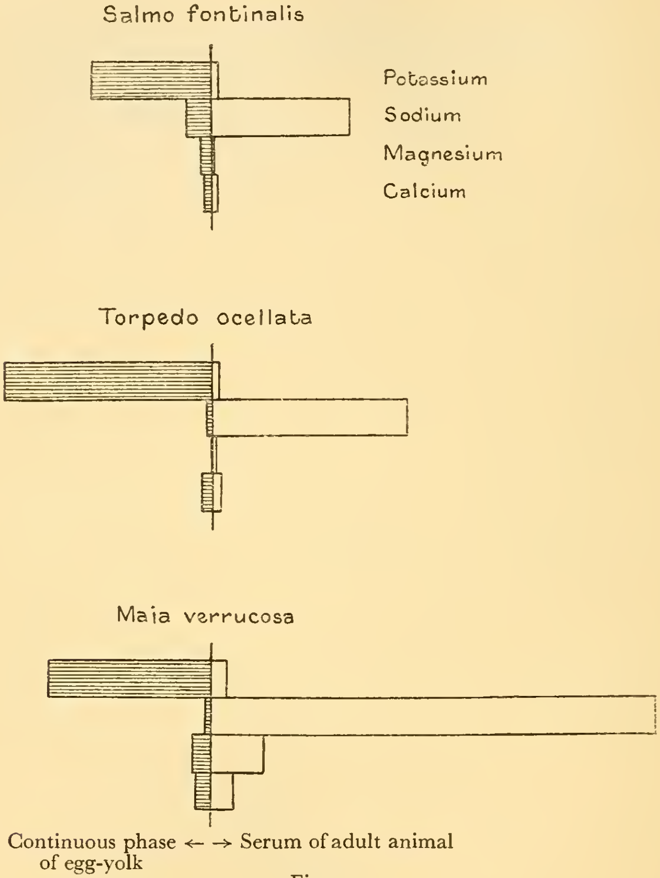

Fig. 20.

tein. The result came out to $10{\cdot}4 \times 10^{-3}$. If this work were repeated for the yolk, interesting commentary on Bialascewicz's researches would be possible. It agrees with the earlier measurements of Bellini, who found the electrical resistance of the unincubated white to be Ω 18·8 ohms. Much further work on such properties of the yolks and egg-whites of a wide range of eggs is urgently needed, for they must obviously be of the greatest importance to the developing embryo. Such questions as the electrical conductivity of egg-cells and developing embryos are very relevant here, but must be left for consideration in Section 5.

As a conclusion to this discussion of the chemical constitution of the egg, it may be admitted that great progress has been made in our knowledge with respect to it during the last fifty years. But to a discerning judgment, it remains none the less a matter for great surprise that in view of our comparative ignorance of the chemical architecture of the egg, we know as much as we do about the coming-into-being of the chemical architecture of the finished embryo.

One further matter may be alluded to in this section. The composition of fossil eggs cannot be said to have much embryological interest, but it is hard to exclude a mention of them. The only analyses we have are those of Zoller who worked on the fossil eggs of Chincha Island, off the Peruvian coast, where seagulls have been living and depositing guano from a very remote date. Zoller found that "time, which antiquates antiquities, and hath an art to make a dust of all things" had had that effect on these eggs and had reduced their water content to 14·4 per cent. There was no urea or uric acid present, although the protein had nearly all disappeared and had given rise to ammonium salts. There was no trace of fat or of carbohydrate, and the sulphur of the proteins had all turned into sulphate.

	%
Water	14·4
Cholesterol	0·287
Phosphoric acid	0·045
Total nitrogen	9·45
Ammonia N	8·12
K	14·9
SO_3	16·08

These figures make it only too clear that if palaeontology and biochemistry enter into closer relations than exist at present, it will not be by way of the chemical analysis of fossil eggs. More hopeful approaches will be found in Section 9·15.

SECTION 2

ON INCREASE IN SIZE AND WEIGHT

2·1. Introduction

We have so far been considering the unfertilised egg-cell and its reserves of nutrient material as a physico-chemical system, and we must now proceed to summarise critically what is known about the alteration the egg undergoes in passing into the state of the finished embryo. Subsequent sections will take up the chemical changes during this process in all their complexity, but first the apparently simple phenomena of change of weight must receive consideration. To this undertaking special difficulties are attached; for example, the act of birth or hatching itself, important though it is for the chemical embryologist as the term of his investigations, is yet purely arbitrarily and conventionally chosen as such, and, as far as the organism itself is concerned, may be relatively unimportant. The age at which birth takes place varies in different animals considerably, and may occur earlier or later in development, cutting across cycles of growth at almost any point. However, the study of growth in weight and alteration in shape, is an essential preliminary to the study of the chemistry of the embryo. I do not propose to spend any time in the discussion of definitions of growth.

The actual data which we have concerning pre-natal growth will be found in Appendix I, where they have been placed in the hope that a collection of them will be of assistance to chemical embryologists. No previous assemblage of them has been made, and they are to be found scattered all through the literature. Biochemists have in the past been insufficiently careful to check their results on embryos against normal tables of weight, length, age, etc.

The predecessors of this section are the chapters on growth in d'Arcy Thompson's *Growth and Form* and Fauré-Fremiet's *La Cinétique du Développement.* These authors gave a full criticism of the whole subject, but without special reference to the development of the embryo. Moreover, much has been done since they wrote, and their treatment differs in various ways from what follows here.

It is obvious that the growth of an embryonic organism can be measured in many ways besides that of increasing weight. Its enlarging dimensions in various directions of space can be measured, or its volume, or the quantity of various constituent substances. More will later have to be said about the way in which these different quantities may be thought of as fitting in together and changing with age. But the simplest manner of representing growth will probably always remain the measurement of the increase in weight of the total mass, and it is this which is now to be considered. The relation of this factor to the age and the length of the foetus is a point of capital importance to the chemical embryologist in the knowledge of his material. It is true that the data are fragmentary enough, restricted as they are almost entirely to various mammals and the chick.

2·2. The Existing Data

Cephalopods.

Octopus. A remarkably complete set of data for the embryonic growth of *Sepia* is given by Ranzi, and this is almost all we have as regards invertebrate development.

Insects.

Silkworm. Luciani & LoMonaco have studied the curve of growth through the successive moults in the larval condition, but, in spite of their work and of many other researches on the silkworm larva and egg, I cannot find any in which the increasing weight of the embryo itself has been measured prior to hatching.

Fishes.

Trout. Weighings of fish embryos have been exceedingly few in number, owing to the smallness of their size and the difficulty of separating them from the yolk. Kronfeld & Scheminzki, however, have made some estimations of the increase in weight of trout embryos, and their figures, together with those of Gray, are shown in Table 1 of Appendix 1.

Amphibia.

Frog. In the case of amphibia, where the cleavage in the egg is more or less inclusive of the yolk-laden portion, it is not possible to obtain data for the weight of the embryo itself, for, before hatching, although the protoplasm is constantly increasing at the expense of the yolk, the two elements cannot be separated, and therefore cannot be weighed in isolation. This appears in the figures of Fauré-Fremiet & Dragoiu; Schaper; Davenport; and Bialascewicz, and must always be taken into account when differences between species in water-content and other constants are under consideration, for much confusion may be caused by not distinguishing carefully between yolk plus embryo and embryo alone.

Reptilia.

Snake. Bohr's very few figures on *Coluber natrix* are all that are available. (Appendix I, Table 2.)

Birds.

Chick. It is on this animal, as might be expected, that the greater part of the work on embryonic growth has been done. Hasselbalch, in the course of his work on the respiration of the chick embryo, obtained a regular series for a race not given. These corresponded well enough with the earlier data of Falck (also from an unknown breed), which were the first to be published, appearing in 1857. Hasselbalch's curve is shown in Fig. 21, in which for the first time we see the usual "embryo-placenta relation" in the form of a weight of extra-embryonic structures larger than the embryo in the earliest stages, but soon falling below it.[1] Hasselbalch calculated the relation between the two as follows:

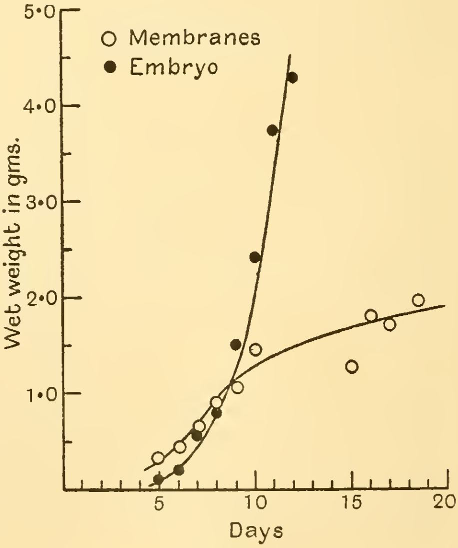

Fig. 21.

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[1] See also Fig. 521.

Day	$\frac{\text{Wt. of embryo} + \text{wt. of membranes}}{\text{Wt. of embryo}}$
8	1·917
9	1·652
10	1·613
15	1·108
16	1·128
17	1·110
18	1·090

Other sets of weight data have been reported by Lamson & Edmond, by Murray and by Needham, for White Leghorn chicks, and by LeBreton & Schaeffer for chicks of an unspecified race. These are all placed in Table 3 of Appendix 1, where it will be seen that the general agreement between them is good. The values obtained by Murray; Byerly, and Schmalhausen are probably the most accurate, for the conditions were very carefully controlled. Hanan's values are lower than all the others. It is unfortunate that Schmalhausen does not state what breed of hen was used in his experiments, though he does mention that it was not genetically pure. Some early measurements by Welcker & Brandt are not included in the table, for they do not appear to be trustworthy.

Other measurements which are useful are those of Edwards, who has published a peaked curve showing the length of the primitive streak during the first 50 hours of incubation.[1] Schmalhausen's work on the growth of parts of the chick embryo will be dealt with later: he was preceded by Falck, who measured and weighed various organs but did not use enough material to make his figures valuable to modern workers.

Mammals.

(*a*) *Mouse.* In 1923 LeBreton & Schaeffer published figures for the embryonic growth of the mouse, but these were not very numerous. The only other work on this subject is that of McDowell, Allen & McDowell, probably the most accurate and satisfactory study of prenatal growth in any form that at present exists. Their figures are given in Table 4 of Appendix 1, and the curve obtained from them in Fig. 22. This is drawn on arithlog paper, the ordinates in logarithmic ruling giving the actual weights in gm., the abscissae in arithmetical ruling giving the age *and* the number of the individuals. On each day the range of the individual unclassified weights is shown by a vertical line which is itself used as a base-line for the frequency distribution of the classified individual weights. The number of cases in the distribution is shown by the distance to the right of the vertical base-lines, and can be judged by the frequency-scale at the bottom of the

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[1] See also Fischel and Levi.

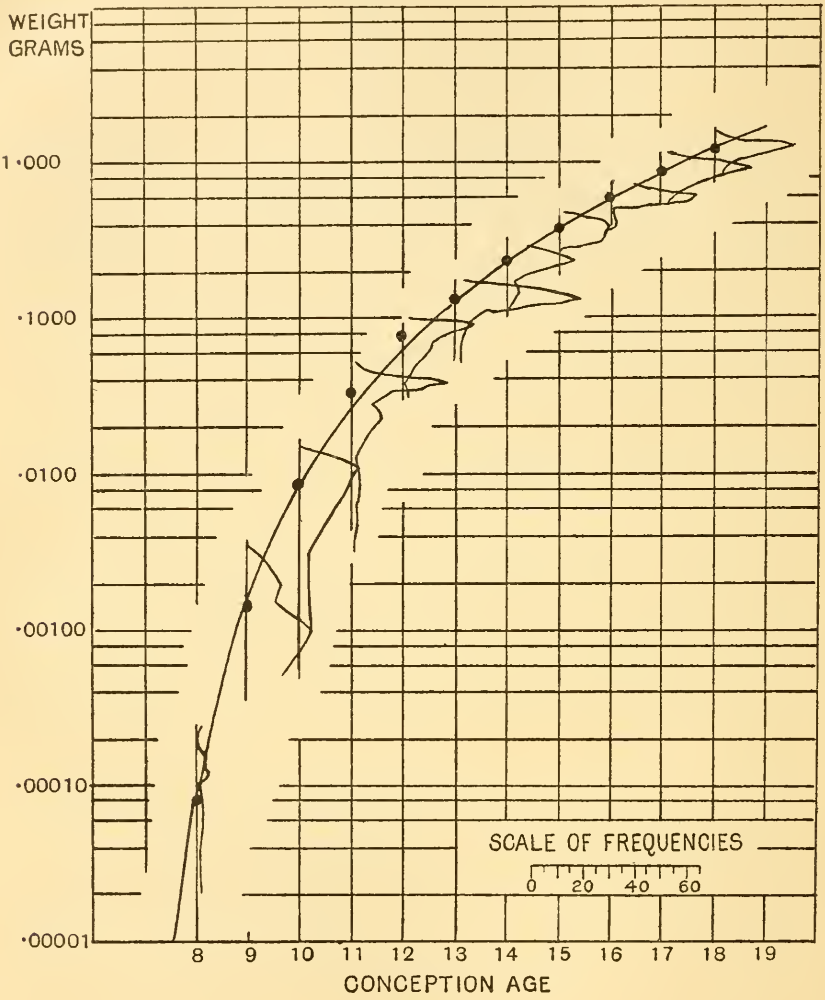

Fig. 22.

chart. The means, weighted by the number of individuals in each litter, are shown as dots on the vertical base-lines, and it is through these, of course, that the "normal curve" would be drawn. The continuous curve in the graph is one drawn to a formula which will be discussed later, in Section 2·4 (p. 393). The lay-out so made reveals several interesting features; it appears, for example, that there are always individuals on a given day which are equal in weight to the mode of the day before. McDowell, Allen & McDowell consider that this is evidence of a possible delay of as much as 24 hours between copulation and fertilisation, but, whether this is so or not, it certainly equates with exactly similar variations found in the chick both in the early stages (primitive streak) and in the later ones of organ-growth. Further, the modes and means are generally close together, though less so at the beginning of development than at the end, and the latter do not approximate to a straight line. A glance at the graph also shows that the highest individual weights on each day tend to form a curve parallel with that of the means throughout development.

(*b*) *Rat.* Donaldson's comprehensive monograph of 1915 includes a discussion of the growth of the rat embryo, but much less work has been done on this animal than upon man, for in the latter case the *ad hoc* labours of obstetricians have often provided much valuable material for the biologist. However, Stotsenberg's work gave a good account of the matter, and his figures are reproduced in Table 5 of Appendix I, and in Fig. 23. They begin from the 13th day after insemination, before which weighing is difficult, and they continue until birth, which takes place at the 22nd day. This pre-natal period would appear to be one complete growth-cycle, if we may judge from the work of Donaldson, Dunn & Watson on the post-natal growth of the rat.

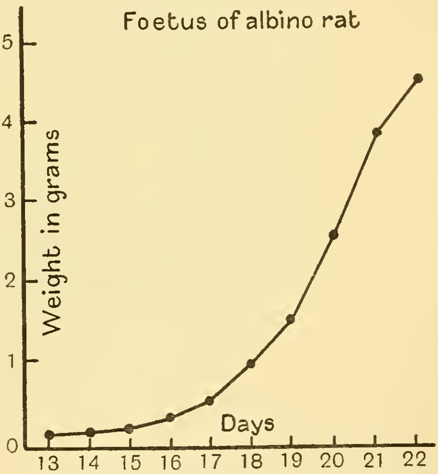

Fig. 23.

Huber has studied the growth of the rat embryo in its earliest stages prior to fixation to the uterine wall. He states that the egg-cell of the rat approaches the uterine end of the oviduct while in the two-cell stage, segmentation being slow and proceeding as the transit takes place. Fig. 24, reproduced from his monograph, is a photograph of a model of the oviduct with its contained eggs. By reconstruction methods at a magnification of 1000 diameters of the ova Huber was able to determine the volume changes during segmentation as follows:

Age			
Days	Hours	Stage	Average vol. (cubic mm.)
1	0	Pronuclear	0·000156
2	0	2-cell	0·000162
3	1	4-cell	0·000173
3	17	8-cell	0·000184
—	—	11-cell	0·000210

There would, therefore, appear to be a certain increase in volume during these very early stages, but as the specific gravity changes are not known it is difficult to understand what it may imply. There is at present a great gap in our knowledge of the embryonic growth of the rat between the early point at which Huber's studies end and the later one at which those of Stotsenberg begin. Huber himself suggested that the slow development of the ovum of the rat during its passage down the oviduct was best accounted for by the lack of any food-supply for an alecithic egg until fixation to the uterine wall had taken place. As the whole embryonic period of the rat is only 22 days, it is of great interest that the first four days should involve hardly any increase in size. This fact renders of no significance the calculated weights of rat foetuses given by Donaldson, Dunn &

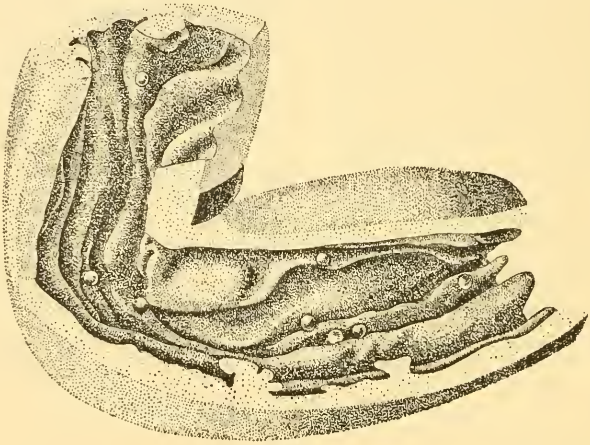

Fig. 24.

Watson in their earlier paper, for, in assuming that embryonic growth in the rat followed a quite similar course to that taking place in man and the rabbit, they did not allow for the long time taken for the rat egg to pass through the oviduct after fertilisation. Thus they arrived at the result that the rat embryo of 15 days should probably weigh 2·610 gm., whereas by direct measurement Stotsenberg found that it only weighs 0·168 gm. Their calculated figures are consequently not included in Appendix I.

(*c*) *Guinea-pig.* The most usually quoted work on the embryonic growth of this animal used to be that of Read, who used a very indirect method of measuring it. He weighed the pregnant female every day between insemination and birth and then each foetus with its membranes and fluids, from which data, assuming that growth had taken place regularly, the weight of one embryo could be calculated. He concluded that the guinea-pig passes through two growth-cycles during its intra-uterine life. But no satisfactory conclusion can really be drawn from such figures, subject as they are to all kinds of complicating factors, and, like the earlier ones of Minot on the guinea-pig, obtained in the same indirect way, they are better discarded. It is needless to point out that differences in the weight of mother + embryo due to defaecation, filling of caecum, etc., may amount to grams, while the weight of the embryo is still only milligrams. In the absence of any other figures, they had their importance, but in 1920 Draper made a complete study of the embryonic growth of the guinea-pig. Together with the few fragmentary (but direct) figures of Hensen, and the careful work of

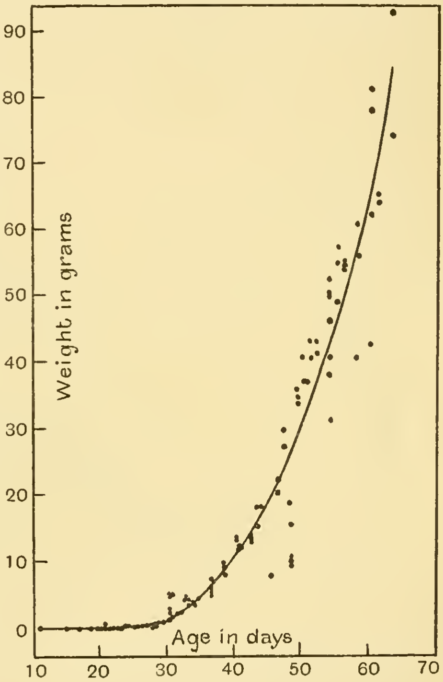

Fig. 25.

Ibsen, and Ibsen & Ibsen, Draper's figures form the standard series, and are shown in Tables 6 and 7 of Appendix I. As is generally known, the guinea-pig differs from most other mammals in being born much later in its life-span than is usual, so that its lactation period is exceedingly short and it is able to eat green food a very few days after its birth. This is reflected in its gestation time which is relatively long.

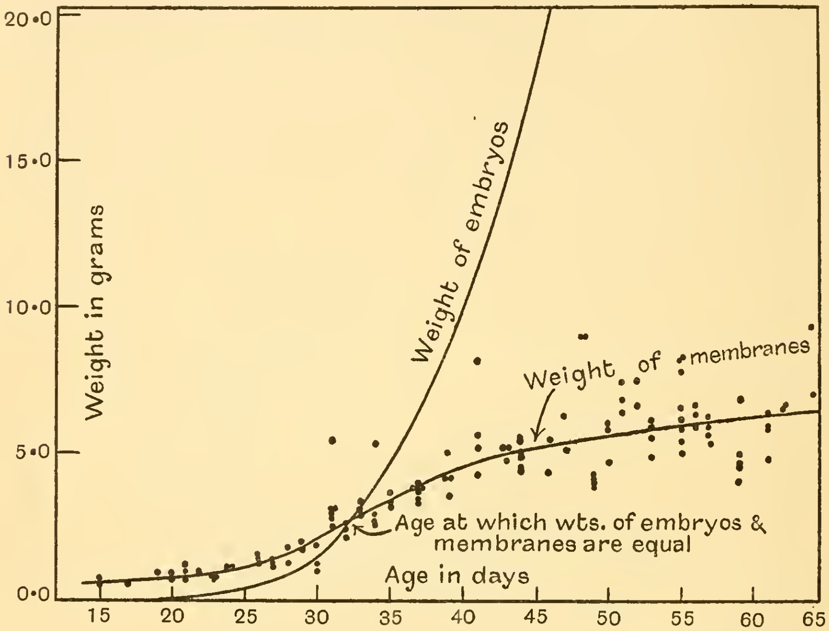

Fig. 26.

During the 64 days of its development *in utero*, the guinea-pig increases its weight to about 85 gm. and its length to 10 cm. This process is shown in Fig. 25 taken from the figures of Draper. In Fig. 26, which gives an enlarged view of the lower part of the growth-curve, the increase in weight of the placenta, the membranes, and adnexa, together with the amniotic and allantoic fluid, is also shown. The extra-embryonic structures reach a more or less constant weight about two-thirds of the way through development, but, as can be seen from Table 5 of Appendix I, the values from which this curve was drawn are very divergent. In comparing the growth of the embryo with the growth of the membranes, it is interesting to see that for the first month the latter weigh much more than the former, after which, for a certain period, they grow together at the same rate. But soon the curves diverge, and the membranes hardly grow any more, while the embryo continues to increase greatly in size. Evidently when the membranes and placenta have reached a sufficient size to meet the utmost further demands of the embryo they grow no more. There can be little doubt that the size of the placenta exercises an influence on the growth of the embryo, and is of the highest importance from the point of view of embryonic nutrition.

The amniotic liquid bears the same relationship as regards weight to the embryo as do the placenta and the membranes.

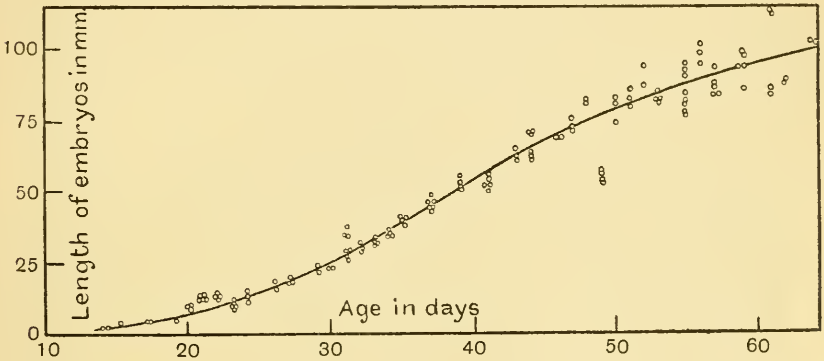

Fig. 27.

Fig. 27 shows Draper's curve for the length of the embryonic guinea-pig.

Ibsen's work led to much the same conclusions as regards the relations between embryo and adnexa as that of Draper. Ibsen found that the number of foetuses in the uterus exerted an effect on the growth-rate of each one, thus the larger the litter the slower the rate of growth of the individual foetus. The early growth of the placenta is more rapid than that of the foetus, but they reach the same weight on the 25th day, after which the foetus outstrips the placenta very soon. Placental weight and the weight of the membranes towards the end of pregnancy are closely correlated with uterine crowding, but this is not the case with the *decidua basalis*, which corresponds to the maternal part of the placenta. Minot considered that the amniotic fluid of the cow and of man decreased in amount after the middle of pregnancy, but this was not found to be the case by Ibsen for the guinea-pig. Ibsen constructed from his experimental data the interesting diagram shown in Fig. 28, which shows the percentage of the whole system occupied by embryo, placenta, decidua basalis, and amniotic fluid from the 20th day onwards. The embryo does not rise in per cent. after the 55th day, the placenta remains very much the same all through, the decidua basalis is much smaller relatively at the end than at the beginning and so is the amniotic fluid. Up to the 50th day Ibsen found no correlation between foetus-weight and placenta-weight, but afterwards there is undoubtedly such a correlation, evidently due to crowding.

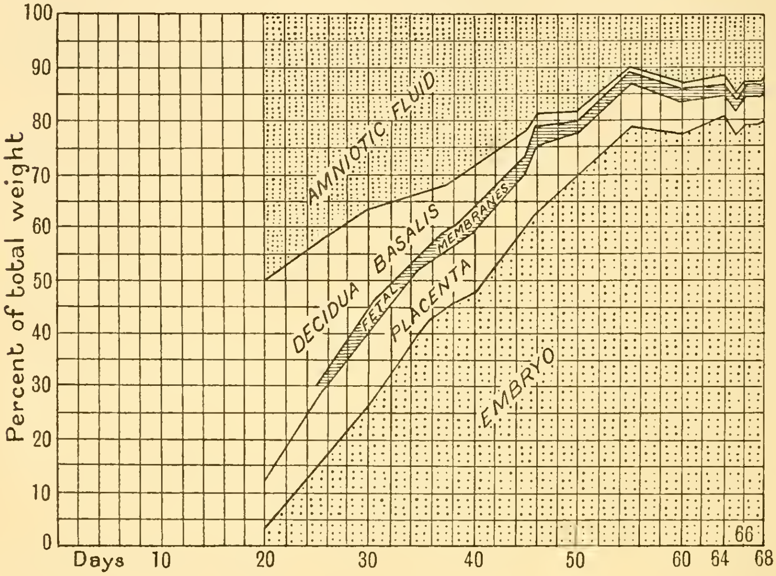

Fig. 28.

(*d*) *Rabbit*. Much less work has been done on this form than might have been expected from its easy availability, but the figures of Chaine (the standard ones) are given in Table 8 of Appendix I, together with some early fragmentary ones of Fehling. Friedenthal also gives a few data which are shown in Table 9.

(*e*) *Dog*. Liesenfeld, Dahmen & Junkersdorf made a thorough study of (unfortunately only 5!) dog foetuses.

(*f*) *Sheep.* As early as 1847 Gurlt made a study of the increase in length of the foetus of the sheep, but Colin is the only investigator who has ever determined the growth in weight (see Fig. 29). Gurlt's figures, which are quite regular, are given in Tables 10 and 11 of Appendix I. Fauré-Fremiet & Dragoiu, in the course of their extended work on the growth and chemical development of the embryonic lung in the sheep, made measurements of the growth of that organ, but did not give any data on the weights of their foetuses as a whole, a very unfortunate omission in view of the incompleteness of the literature on this subject.

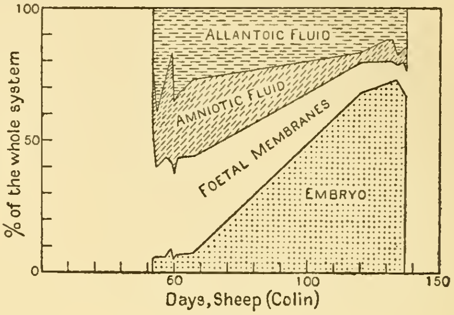

Fig. 29.

(*g*) *Pig.* The only extensive figures in existence for the embryonic growth of the pig are due to the careful work of Lowrey and of Warwick. These are given in Tables 12 and 14 of Appendix I. Lowrey's results will again be referred to in connection with the growth of individual organs and parts in the embryo. LeBreton & Schaeffer also measured and weighed a certain number of foetuses in the course of their classical work on the behaviour of the chemical nucleo-plasmatic ratio during embryonic development. Their figures are shown in Table 13 of Appendix I.

(*h*) *Cow.* The embryonic growth of the cow has been investigated by several workers whose results are shown in Table 15 of Appendix I.

Fig. 30, constructed from Franck and Hammond, should be compared with Fig. 28 for the guinea-pig.

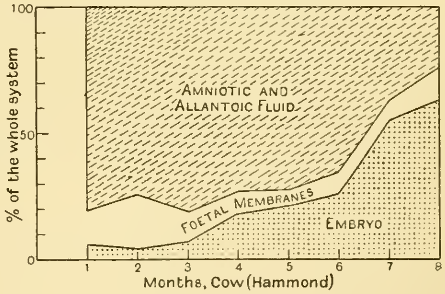

Fig. 30.

(*i*) *Man.* The embryonic growth of man has been much studied, and many thousands of embryos have been weighed. His's studies have been the principal means of fixing the relation age/length, and Balthazard also gives figures for this, which will be found in Appendix I (Table 16). The earlier workers, Ahlfeld; Fehling; Hennig; Legou; Faucon; and Michaelis all obtained valuable data, but it was not until 1909 that a critical examination of them was made by Jackson who analysed the figures of his predecessors, and added a large number of new ones. His results gave a continuous curve from the earliest stages till birth, which agreed with the majority of the other investigators, but not perhaps with Hennig's curve (he gave no figures), which showed a very distinct slackening of growth about the sixth month, after which the same rate was resumed. If this phenomenon is real, it may possibly be associated, as Donaldson has suggested, with a transition from one growth-cycle to another, at the end of the sixth month, when the absolute weight begins to rise so rapidly. On the other hand, the mass of data which Quetelet and others after him have analysed regarding the growth of man throughout life, would seem to show that there are three growth-cycles only, one pre-natal one, one with its maximum at 5·5 years, and the third with its maximum at 16 years. Vignes' S-shaped curve for human embryonic growth is shown in Fig. 31.

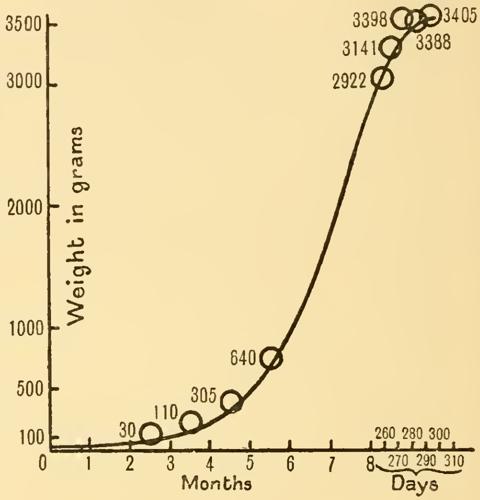

Fig. 31.

Bujard in 1914 made a geometrical analysis of the early stages of the human embryo.

Jackson measured the volume and weight of all the specimens in his own collection, and for the early stages also the volumes of the His-Ziegler models. His figures are given in Tables 17 and 18 of Appendix I, and the curve which he constructed from his own data as well as those of previous investigators is reproduced in Fig. 32. The 16th table of the appendix shows the volumes of the His-Ziegler models, and demonstrates that the human embryo, like all others, is much exceeded in size by the yolk-sac during the earliest stages of development. Jackson, who adopted the Minot method of measuring the growth-rate, concluded that the rate was 9999 per

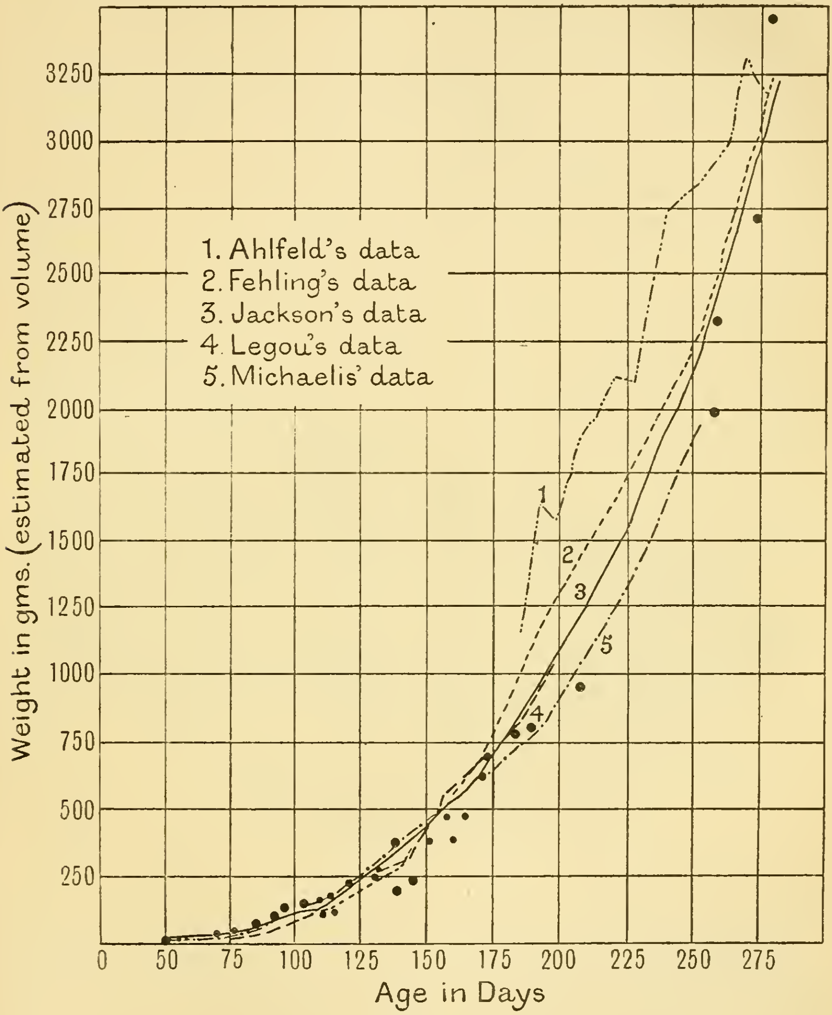

Fig. 32.

cent. for the first month, 74 for the second month, and 11 for the third month. This was in general agreement with the point of view taken by Mühlmann, and Jackson emphasised it further by showing that, if the weight of the embryonic membranes and fluids was taken into account, the growth-rate for the first month was 574,999 per cent. What meaning can be attached to the enormous growth-rate figures which always appear when the Minot method is used for very young embryos must later be discussed.

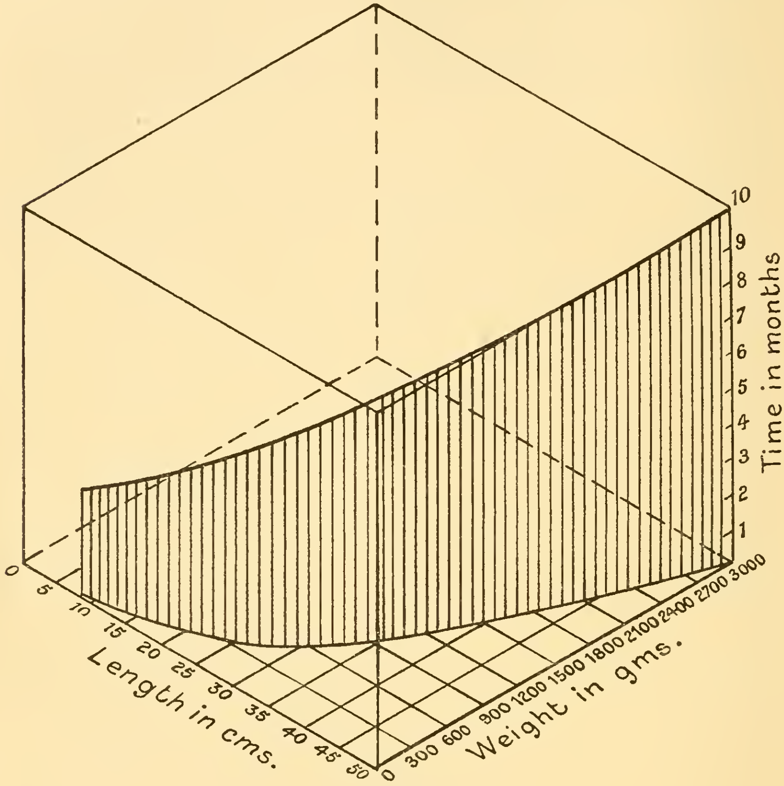

Fig. 33.

Fig. 32, which collects together the data of many observers, shows a considerable measure of unanimity between them. Ahlfeld's figures are the only ones which show serious divergence, and they were not taken into account by Jackson in his preparation of the "normal curve". Fig. 32 shows also by points the volume of the embryo at the different stages, but it does not differ much in value from the weight in grams. The specific gravity of the foetus does not, according to Jackson, remain precisely the same throughout development, but changes from very slightly above 1·0 in the early stages to 1·05 in the later ones. Probably this is associated with the progressive loss of water as the embryo develops.

One of the first to investigate quantitatively the growth of the human embryo was Boyd in 1861, who studied the weights of all the principal organs in embryos from 8·5 to 85 oz. He did not give figures for individual embryos from which a curve could be constructed, but simply divided them into large groups such as "prematurely-born", etc. Legou's data, already referred to, were worked over again in 1903 by Loisel. Zangemeister, more recently, has published figures for human embryonic growth—these are shown in Table 17 of Appendix 1.

Other data for embryonic growth in man will be found in the papers of Fesser; Toldt; Meyer; Heuser; Bedu; Sombret; Arnoljevic; Stratz; Borri; Corrado; Balthazard & Dervieux; Ecker; Hamy; Kölliker; Cruickshank & Miller; Browne; and Friedenthal. Scammon & Calkins, who have made a great many measurements in recent years, have constructed a three-dimensional isometric projection, from which the height, weight and age of a human embryo may be read off if any one of them is known (see Fig. 33). The best recent paper on the whole subject is that of Streeter.

Sandiford has shown that the weights and surface areas of foetuses fall on straight lines when plotted on double log paper. For further information on surface growth see Scammon & Klein.

(*j*) *Whale.* Some information on the embryonic growth of the whale is contained in the papers of Harmer; Risting; Hinton; and McIntosh & Wheeler, but it mostly concerns increase in length.

2·3. The General Nature of Embryonic Growth

We may now turn to the theoretical aspect of the matter in the attempt to find out what interpretation can be placed upon them. We may in the first place take as a simple example of an embryonic growth-curve the work on the growth of the chick (White Leghorn) of H. A. Murray. Table 51 shows, firstly, the actual weights of the embryos on each successive day, secondly, the amount gained in each such 24-hour period, i.e. the amount of substance actually added on to itself by the embryo during the lapse of the time in question. This is known as the daily increment. In the next column the averages of the daily increments are placed, and these figures, known as the mid-increments, represent for each point which begins one period and ends another how much substance the embryo is adding on to itself between the times (*a*) half-way through the last period, and (*b*) half-way through the period to come. In other words, the mid-increments convert the daily increments into terms of the points instead of the spaces between the points. If now the mid-increments are expressed as percentages of their actual weights of embryo at their corresponding points in time, the last column or percentage growth-rate is obtained. This last calculation is, as will be seen, the only one so far made in the table which is open to serious criticism, and it is associated with the name of C. S. Minot, who was the first to propose it as a satisfactory measure of the growth-rate of an organism. When these figures are plotted the curves shown in Fig. 34 appear. The actual growth of the embryo expressed in terms of its

Table 51. *Growth of the chick embryo (White Leghorn).*

H. A. Murray's figures.

Age in days	Wet weight (mg.)	Dry weight (mg.)	Daily increment (dry weight)	Mid-increment (dry weight)	Percentage growth-rate of dry substance
5	221	11·75			
			11·85		
6	423	23·6		15·7	66·5
			19·4		
7	735	43·0		25·1	58·4
			30·8		
8	1,189	73·6		37·5	50·8
			44·3		
9	1,817	118·1		56·2	47·5
			68·2		
10	2,661	186·3		85·3	45·7
			102·5		
11	3,750	288·8		131·6	45·6
			160·7		
12	5,105	449·5		200·8	44·7
			241·0		
13	6,839	690·5		325·2	47·1
			409·4		
14	8,974	1099·0		492·0	44·7
			575·0		
15	11,460	1674·0		630·0	37·6
			686·0		
16	14,390	2360·0		708·0	30·0
			730·0		
17	17,950	3090·0		763·0	24·7
			797·0		
18	22,030	3887·0		814·0	20·9
			832·0		
19	26,670	4719·0			

weight at any given moment gives a curve which rises steadily till the observations cease without betraying much sign of any slackening. But the increment curves, on the other hand, show an unmistakable **S**-shape which is due to the fact that, for the earlier periods, the

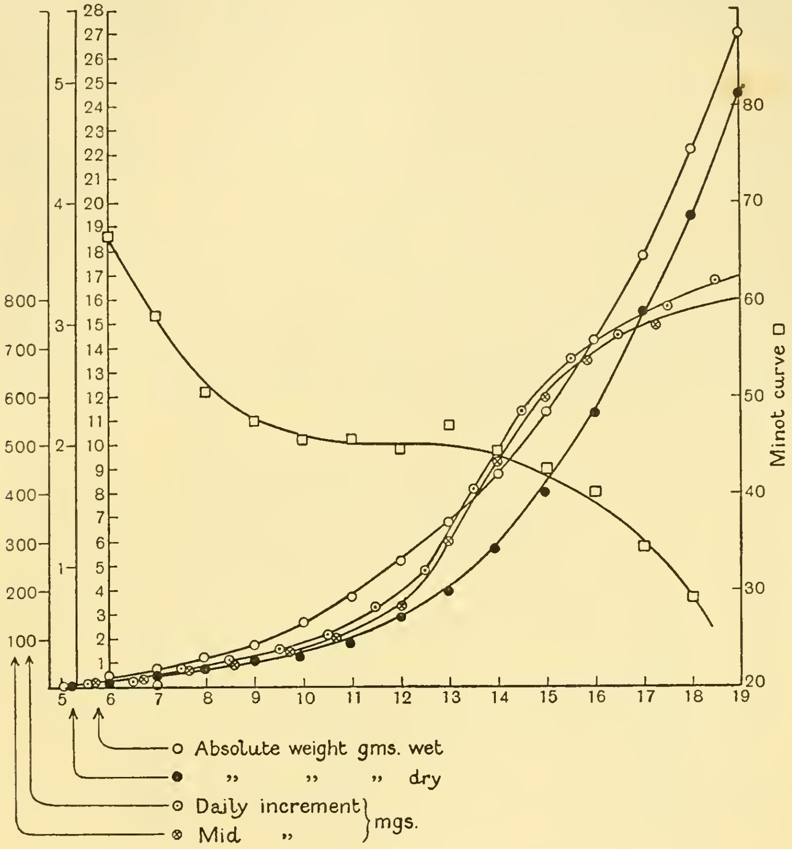

Fig. 34.

weight gained each day is very little more on one day than the gain on the previous day, while, towards the middle of development, the daily increments and the mid-increments vary much more, each one being considerably higher than the one before. On the other hand, when the end of development is approaching, the increments each day, though far higher in absolute amount than those which were made in the early stages, do not differ so materially from one another, with the result that the curve slackens off and enters a slowly-rising phase again. It is possible, of course, to calculate the average daily increment for the whole developmental period (see Table 52), and the figures so obtained have been made the basis of a comparison of animals by Friedenthal. The fourth curve, that of the percentage growth-rate, Minot's curve, as it may be called, begins at a high level and continually descends, although in this instance there is a slight kink on it midway through development, which may for the moment be disregarded. All Minot curves begin at a high level and descend as development proceeds. Now, in many cases, it may happen that not only the increments but also the whole growth-process itself slackens off towards the end of the period taken, in which circumstance the curve relating weight of animal at any given time to age will also have an **S**-shaped form. It has not escaped the perspicuity of those who have considered these phenomena that this **S**-shaped curve has a resemblance to the **S**-shaped curve of an autocatalysed monomolecular reaction, and this likeness will shortly be taken up at length.

Table 52. *Average daily increments.*

Friedenthal's figures.

	gm.		gm.
Mouse	0·08	Pig	14
Rat	0·24	Man	15
Ermine	0·3	Sheep	26
German marmot	1·2	Seal	30
Musk	1·5	Ass	53
Guinea-pig ...	2·0	Rhinoceros ...	90
Wolf	4	Stag	100
Puma	5·4	Horse	200
Bear	7·0	Hippopotamus...	200
Lion	10	Camel	400
Roedeer	11	Elephant ...	400

A more complicated example of the various types of growth-curves is afforded by Fig. 35 taken from Brody. It shows the growth throughout the life-span of the albino rat. The curve passing through the circles shows the course of growth; it is, in fact, the weight of the whole animal at any given moment plotted against the age at that moment. The strongly indented curve, passing through the crosses, is the line showing increment in unit time. In the data of Murray for the embryo chick the absolute growth-curve rises steadily, and has no slackening off or self-inhibitory phase; the increment curve is therefore singly sigmoid. But here, when the absolute growth-curve is itself sigmoid, the increment curve is symmetrically sigmoid, rising to a maximum and then falling away again to zero during the second phase. Finally, the corresponding Minot curve is shown by the line joining the triangles, and, as usual, it declines throughout growth from an initially very high value.

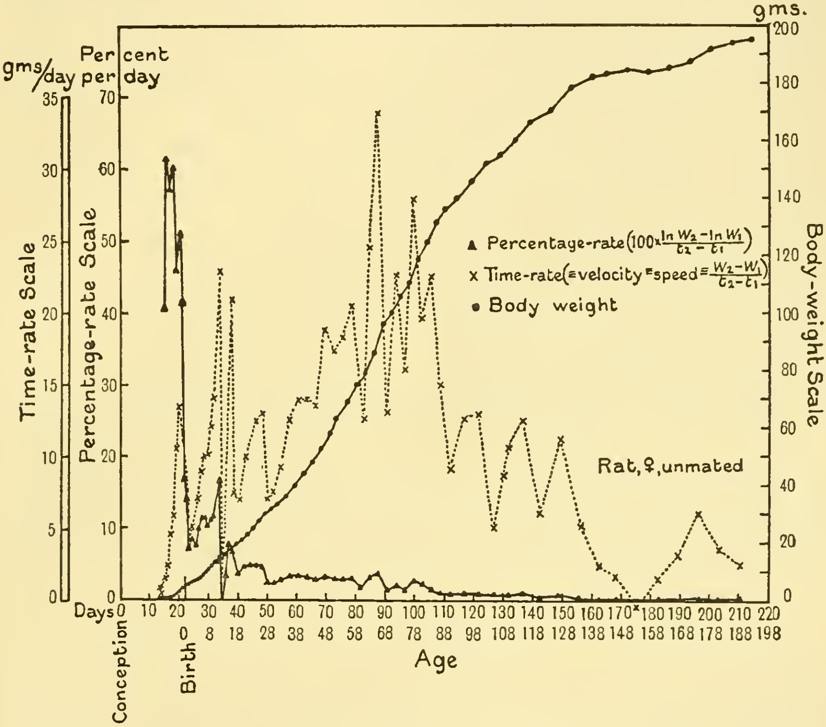

Fig. 35.

There are other ways, however, in which the subject of embryonic growth-curves can be introduced. Ostwald's classical work on growth in metazoa, which appeared in W. Roux's "Vorträge" series in 1908, laid great emphasis on the value of knowing the precise route through weight taken by an organism on its way from egg-cell to finished embryo. In Fig. 36, taken from his monograph, several different curves are shown relating time to weight. At the time *A*, at hatching or birth, for instance, the weight of the organism is exactly the same in all four cases, but the manner in which the increase in weight has taken place is in the four cases profoundly different. It is certainly quite clear that the chemical embryologist, engaged in the attempt to understand the processes which contribute to the final result, must pay detailed attention to the path by which this final result is arrived at. The four different curves in Ostwald's figure would imply four very different sets of conditions within the developing embryo. An embryo which grew according to Curve I would change very rapidly in the beginning, and afterwards change progressively less rapidly as the curve became asymptotic. The reverse of this process would happen if the embryo grew according to Curve II, for there the process continually increases in rapidity, and is proceeding at its fastest when the point *A* is reached. Curve III, on the other hand, being S-shaped, would seem to indicate the presence of an autocatalytic process, for at first the growth proceeds faster and faster, but later on, after the point of symmetry of the curve has been reached and passed, slower and slower. Several such S-shaped curves superimposed on one another make up Curve IV.

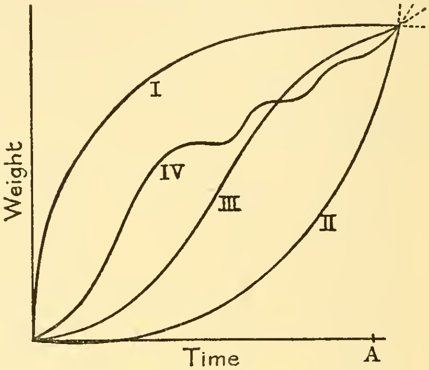

Fig. 36.

As far as is known, no growth takes place in the manner represented by Curve I, but rather in that of the other three curves, though our present knowledge does not enable us to say definitely which, except in certain cases. Ostwald's monograph should be referred to by those who wish to see how he continued the discussion of growth-curves, for it is probably the best presentation of the subject, and it was certainly written from a much less doctrinaire point of view than most of its successors.

The general interpretations of embryonic growth-curves may be divided into several classes. They depend more than anything else, as will be clearly seen, upon how the facts are expressed. One way of expressing them led Minot to his "laws of cytomorphosis", another led Robertson to his "autocatalytic master-reaction", and, more recently, still other ways have been devised. The unprejudiced investigator cannot avoid a considerable measure of scepticism in considering the claims of one way of expressing the facts over another.

2·4. The Empirical Formulae

We may first direct our attention to those presentations of the facts which do not carry with them any theoretical superstructure, but aim simply at describing the data in as short a manner as possible. The first of these "empirical formulae" was that of Roberts, who in 1906 pointed out that the growth of the human foetus could be regarded as nearly proportional to the cube of the age; thus, if the weight in grams is W and the age in days T, the formula would be $W = T^3$. But this was only very approximate, and the curve it gave did not fit the curve drawn through the experimental data with any accuracy. Roberts, indeed, stated that his formula gave results correct to "within an ounce at the third month". "Since the weight of an embryo of the third month," was Meyer's remark, "according to the best available evidence, is considerably less than an ounce, the accuracy of Roberts' method must be fully apparent without further comment."

Tuttle next introduced an equation in which arbitrary constants were introduced, thus $W = 50\,(T - 2)^2$. Later still, Jackson, whose work on the human embryo has already been mentioned, proposed the formula:

$$W = \left(\frac{T}{37}\right)^4,$$

where W is the weight in grams and T the age in days. This fitted the experimental points much better than the formulae of Roberts and Tuttle, but was still rather deficient, especially in the very early period and the very late period. Henry & Bastien also proposed

$$x^2 + 25xy - 30y^2 - 162y = 0,$$

where x = months and y = kilos.

Duvoir has reviewed the other more or less practical rules which have from time to time been proposed, such as Casper's rule that, from the fifth month onwards, the age of an embryo in man can be found by dividing the height in centimetres by 5. Balthazard & Dervieux altered this formula to 5·6. Again, Mall's rule states that the number of days embryo age is equal to the square root of the foetal total length in centimetres × 100. Balthazard & Dervieux have also evolved formulae relating foetal age to the length of the limb-bones, e.g.:

$$L = \text{femur length} \times 5{\cdot}6 + 8 \text{ cm.}$$
$$L = \text{humerus length} \times 6{\cdot}5 + 8 \text{ cm.}$$
$$L = \text{tibia length} \times 6{\cdot}5 + 8 \text{ cm.}$$

The use of empirical formulae in the description of human foetal growth has been carried to its greatest refinement in the work of Scammon & Calkins, whose formula,

$$T = 2{\cdot}3 + \frac{2{\cdot}5L}{28} + \frac{L^2}{784},$$

holds with great exactitude from the third month onwards. Another of their formulae,

$$T = 2{\cdot}134 \times 0{\cdot}1L \times 0{\cdot}0011L^2,$$

holds with rather less exactitude from 2·5 foetal months onwards. In both these cases, T is the menstrual age in lunar months, L the total or crown-heel length of the dead body in centimetres. They also found that

$$W = (0{\cdot}26L)^{3{\cdot}108} + 4{\cdot}6,$$
$$\text{or } L = 3{\cdot}846 \sqrt[3{\cdot}108]{W - 4{\cdot}6},$$

where W is the weight of the dead body. From these equations, it follows that

$$T = 2{\cdot}134 + 0{\cdot}3846 \sqrt[3{\cdot}108]{W - 4{\cdot}6} + 0{\cdot}01627 \sqrt[1{\cdot}554]{W - 4{\cdot}6},$$
$$\text{or } T = 3{\cdot}0 + 4{\cdot}049 \sqrt{W - 0{\cdot}012},$$
$$\text{or } W = 0{\cdot}561 - 0{\cdot}366T \times 0{\cdot}061T^2.$$

The formula of Donaldson, Dunn & Watson, for the post-natal growth of the white rat up to 80 days, $W = a + bT + cT^2$, and after 80 days, $W = a \log T - bT - c$, was of the same type as the other equations mentioned, but it had the additional refinement of including constants, a, b and c, which were variable according to sex and other factors.

Murray, in his study of the chemistry of embryonic development, found that his series of chick embryo-weights could be accurately described by the equation:

$$W = \frac{T^{3 \cdot 6}}{1 \cdot 496},$$

$$\text{or } W = KT^{3 \cdot 6},$$

where $K = 0 \cdot 668$.

This was not unlike the Balthazard-Dervieux formula for the human embryo:

$$T = 19 \cdot 4 \times \sqrt[3]{W}.$$

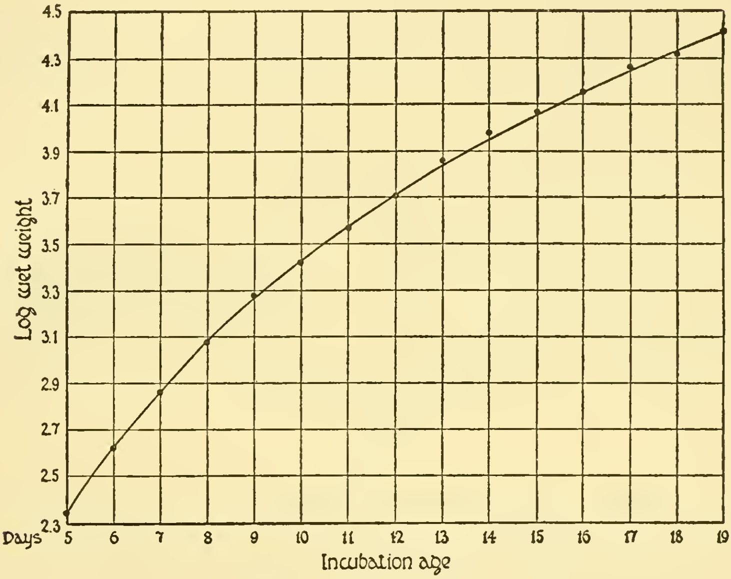

Fig. 37.

Murray plotted the log. wet weight against the incubation age, and obtained a curve concave to the abscissa (see Fig. 37) corresponding to the curve which McDowell, Allen & McDowell got for the mouse embryo (see Fig. 22). He also found that the relation of log. weight to log. age was a straight line as far as his series of weighings went, and showed that the weighings of Hasselbalch and of Lamson & Edmond fell on the same straight line (Fig. 38). Murray's formula gave very good agreement with his figures, but these did not extend further back than the fifth day of incubation. When, later, Needham and Schmalhausen made weighings of embryos between the second and the sixth day of incubation, it was found that Murray's formula did not hold for these earlier stages. Fig. 39 taken from the paper of McDowell, Allen & McDowell, illustrates this point. The broken line is drawn to Murray's formula, and the dotted line is an extrapolation of his curve which I made on the assumption that embryos grew at the same rate before 5 days as between 5 and 7 days, i.e. exponentially. The circles with dotted centres are the values experimentally obtained by me, the dots are those obtained by Schmalhausen, and the cross within a circle is Murray's earliest figure.

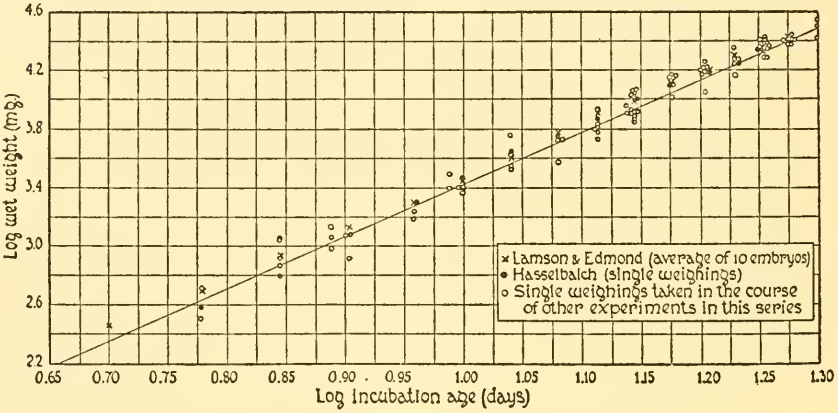

Fig. 38.

Murray's formula gives a line consistently above the experimental points for this early period, and the exponential extrapolation is quite at variance with them. But McDowell, Allen & McDowell evolved an equation which fitted these early points (the solid line) as well as all the later ones, as follows:

$$\log W = 3{\cdot}436 \log \{10\,(T - 0{\cdot}5)\} + \bar{7}{\cdot}626.$$

This new equation was based on an entirely different viewpoint from that of previous workers. McDowell and his collaborators regarded not "conception age" but "embryo age" as the right zero hour to take in growth calculations. It had always been assumed previously that conception or even insemination was the right starting-point, and Brody & Ragsdale and Brody based their method for finding age-equivalence in animals on this view, while Friedenthal had shown a similarity in relative growth-rates by plotting the log. weights against log. conception ages. McDowell and his collaborators, on the other hand, suggested that the time of growth ought rather to be calculated from the time at which the embryo first begins to have an axis, i.e. from the primitive-streak stage. Thus the major differences between the pre-natal growth of the guinea-pig, the mouse, and the chick would be accounted for by the varying times taken to get through the preliminary work of arrangement and organisation. Processes such as gastrulation, according to this view, would involve a law of growth so different from the later axial type that no formula should be expected to cover the two. We have already seen in the case of the rat's egg that some considerable time may elapse between the time of fertilisation and that of fixation to the uterine wall, during which the supply of nutrient material may be

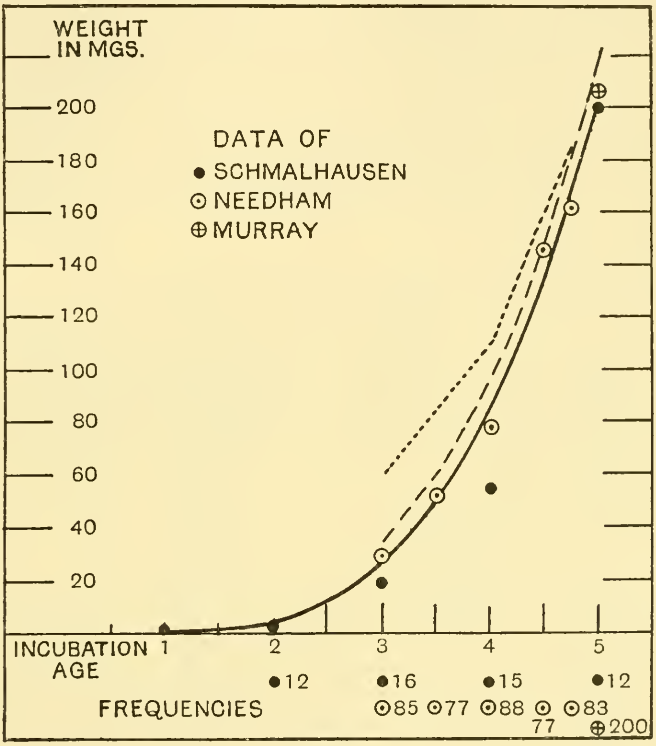

Fig. 39.

very different from what afterwards obtains during embryonic development. There is, therefore, much justification for the view of McDowell and his collaborators. Brody himself had come nearly to this position without recognising it, for, in a paper published in 1923, he had pointed out that, during a short period in the early stages of growth (or regeneration) the apparent observed speed seems to be slower than would be expected. Thus the curve of the fitted equation cuts the time axis not at zero, the beginning of growth, but a little later. He advanced the explanation that Durbin had already applied to the initial slow phase in the regeneration of tadpole tails, namely, that a "cap of embryonic cells" was first formed, following in its growth quite different laws from the subsequent process as a whole. "It is suggested", said Brody, "that the apparent initial slow phase of growth of the individual from the fertilised egg is due to a similar qualitative growth."

(Estimated weights of eggs are shown in Table 53.)

McDowell and his collaborators proceeded to show that a similar formula would fit very well the curves of growth for the guinea-pig (Draper; Hensen; Ibsen & Ibsen), the mouse (McDowell, Allen & McDowell) and the chick (Murray; Needham; and Schmalhausen). For the mouse it was:

$$\log W = 3{\cdot}649 \log \{10\,(t - 7{\cdot}2)\} + \bar{8}{\cdot}6587;$$

and for the guinea-pig it was:

$$\log W = 3{\cdot}987 \log \{10\,(t - 12)\} + \bar{5}{\cdot}1839;$$

The significant thing about these empirical formulae is the deduction of a certain time in each case from the conception age, thus 7·2 days in the case of the mouse, 12 days in the case of the guinea-pig, and 0·5 day in the case of the chick (Allen & McDowell). The evidence on which McDowell and his collaborators rested their case for this shortening of the development time was drawn from various sources; thus, from their own histological observations they found that the primitive streak in the mouse embryo appeared about the 7th day of development, for 6-day embryos show no mesoderm, while 7-day ones do, and usually the primitive groove as well. Sobotta's work is in agreement with this estimate. Their estimate for 12 days as the time taken for the embryo guinea-pig to reach the primitive-groove stage was based on the generally accepted work of Bischoff and Lieberkühn, while, for the chick, Duval, whose illustrations are the usual "normaltafeln", shows the first appearance of the primitive groove at the 10th hour of development, an assessment which is agreed to by Jenkinson and by Foster & Balfour (12th hour).

Table 53. *Probable dimensions of egg-cells.*

		Weight in grams (Friedenthal)	Diameter in μ (Hartmann)
Cyclostomata			
	Amphioxus	0·000001	—
	Lamprey	0·004	—
Pisces			
	Sturgeon	0·004	—
	Pike	0·001	—
Amphibia			
	Frog	0·004	—
Reptilia			
	Crocodile	40·0	—
Aves			
	Hen	45·0	—
	Aepyornis	120000·0	—
Mammalia			
Monotremata	Platypus	—	2500
	Spiny anteater	0·1	3000
Marsupialia	Dasyurus	—	240
	Opossum	0·001	150
Edentata	Armadillo	—	80
Cetacea	Whale	—	140
Insectivora	Mole	—	125
	Hedgehog	—	100
Rodentia	Mouse	0·0000001	72
	Rat	—	72
	Guinea-pig	0·000001	80
Lagomorpha	Rabbit	0·000003	125
Carnivora	Dog	0·000003	140
	Cat	—	125
	Ferret	—	120
Ungulata	Horse	—	135
	Sheep	—	120
	Goat	—	140
	Pig	0·000003	130
Cheiroptera	Bat	—	100
Lemurs	Tarsius	—	90
Primates	Gibbon	—	115
	Macacus	—	115
	Gorilla	—	135
	Man	0·000004	135

"The general course of pre-natal growth in the mouse, the guinea-pig and the chick, can be expressed by straight lines relating the logarithms of the weight and the age only when age is counted from the beginning of the embryo proper." Such is the conclusion of McDowell and his collaborators, and, though it may seem barren in theoretical results, it is nevertheless based on sounder considerations than the more ambitious ones of other workers*. It is probably legitimate to assume that the laws of growth before the formation of the embryonic axis are very different from what they are afterwards. It is also legitimate to assume that the differences between the velocity constants in the three formulae are due to the varying amount of organisation which has to go on in each case before the formation of the primitive groove. Fig. 40 shows the straight-line relationships found to hold by McDowell, Allen & McDowell in the case of the guinea-pig, mouse and chick, and Fig. 41 gives further examples, from which further variants of McDowell's formulae could easily be calculated. Clearly in embryonic growth log. weight is always proportional to log. time.

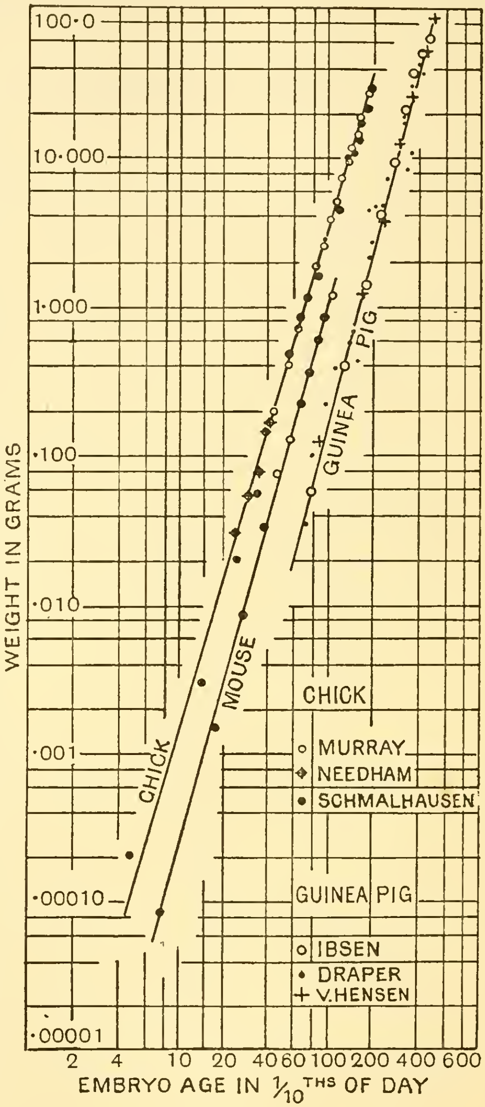

Fig. 40.

With respect to Fig. 39, in which the weights of very young chick embryos are given, it should be noted that the discrepancy would naturally be expected to occur only in the early stages, for in the later ones the difference between conception age and embryo age would be a smaller percentage of the total. The averages for the early embryos reveal the difference by bending away from the lines drawn on the basis of incubation or conception age.

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* But see p. 427.

Schmalhausen, continuing earlier work on the growth of bacilli and protozoa, has also put forward empirical formulae for the embryonic growth of the chick, but his equation

$$\sqrt[3]{W} = T,$$

while fundamentally the same as that of Murray, has no velocity constant. Fig. 42, taken from Schmalhausen's paper, shows that the cube root of the weight plotted against the age only gives an approximately straight line. Schmalhausen has included in the same figure the curves obtained by other methods; thus curve P' is the Minot (percentage growth-rate) curve for the wet weight, and Ps' for dry weight, while the curve marked log $0{\cdot}1p$ is the log. weight plotted against the age. As we have already seen, in the case of McDowell's figures for the mouse, and Murray's figures for the chick, this value always gives a curve rising concave to the abscissa. The curves P and Ps in Fig. 42 represent the absolute wet and dry weights respectively.

Other empirical formulae have been proposed for growth-processes from time to time.

Embryonic growth can be expressed roughly by exponential curves; thus:

$$W = wp^t,$$

where W is the mass of the embryo at time t, w the original mass, and p a constant. Thus the equation of an exponential curve is one in which the power is always changing. Janisch has given a discussion of the use of the exponential curve in all departments of biology, and in it he shows how important this relation is in growth phenomena.

The "law of compound interest" however, put forward by Blackman in 1919 for the growth of plants, and which has been shown by Luyet to be a special form of the exponential relation

$$W = w\,(1 + r)^t,$$

has not so far been of any assistance in describing embryonic growth. Another form of the exponential curve, the arithmetical progression method, which gives the equation

$$\log W = At,$$

where *A* is a characteristic constant, was used by Fauré-Fremiet in 1922 for describing the growth of a *Vorticella* colony, but the conditions there are too far removed from those of embryonic development to make it worth while considering this aspect of the subject in detail. The formula proposed by Fauré-Fremiet for the growth

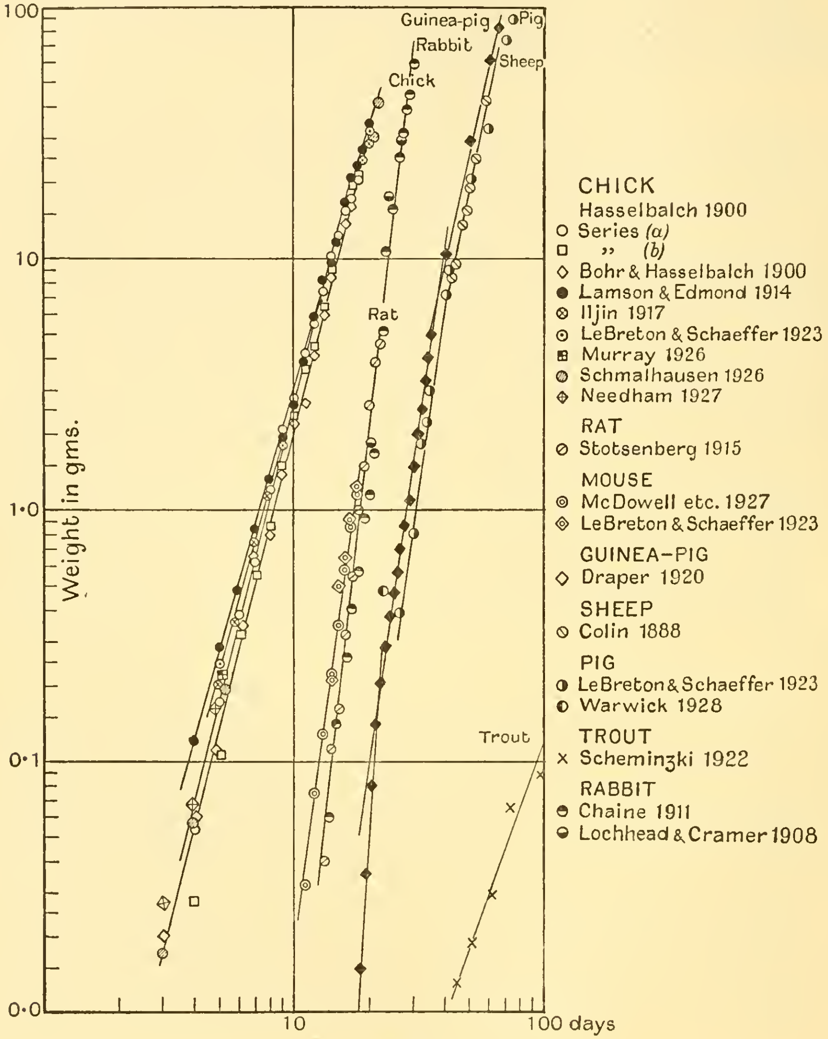


Fig. 41.

of the epithelium of the foetal lung is, however, of greater interest:

$$W = At^3w + Bt^2w + Ctw + Dw,$$

where w is the total weight of the lung at the time in question. But here the number of arbitrary constants is so large that we reach the point where the question naturally arises whether an empirical formula is worth looking for at all. The more complicated it is, the less valuable it is, in view of the fact that, in any case, it is not intended to give us an idea of the basic factors underlying the process.

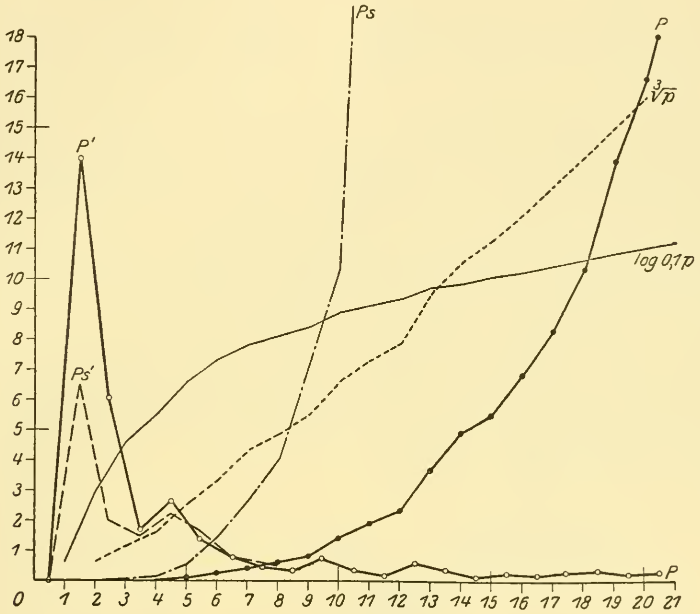


Fig. 42.

2·5. Percentage Growth-rate and the Mitotic Index

We have so far been examining the results of those investigators who have taken the curves obtained by simply plotting the weight of the embryo each day during development against the time, and who have endeavoured to find a correct mathematical expression for them without a preconceived theory. We have now to consider the work of those who have infused more theory into their treatment of the experimental facts.

Before 1890 there was no regularity in the way in which experimentalists examined their data on growth. But about that time Minot began a long series of investigations on the growth of animals, mainly the guinea-pig and the rat, in which he introduced a new method, namely, the evaluation of the growth-rate by taking it as the increment in per cent. of the weight of the animal at the beginning of the period in question. Some workers, e.g. Preyer, had already adopted this plan. The percentage growth-rate has always been found to decline enormously as development proceeds, an observation which led Minot to say that the embryo gets oldest most quickly when it is youngest. This apparently paradoxical statement drew a good deal of attention to his work at the time, and his book, *The Problem of Age, Growth, and Death,* included many such graphs showing how rate of senescence was greatest in the earliest periods. One of these is reproduced here as Fig. 43.

Another contribution of Minot's was the conception of the "mitotic index", or the number of mitosing cells per 1000 cells in a tissue. He did not himself find time to do more than a few of these laborious counts, but he gave the following figures, which showed that the mitotic index declined with age:

Development of rabbit foetus (days)	Tissue	Mitotic index
7·5	Ectoderm	18
	Mesoderm	17
	Endoderm	18
10·0	Ectoderm	14
	Mesoderm	13
	Endoderm	15
	Blood	10
13·0	Spinal cord	11
	Connective tissue	10
	Liver	11
	Skin	10
	Excretory tissue	6
	Muscle	6

These data lent weight to his principal conclusion, which was that the younger the embryo the more rapidly it aged. Practically nothing more was done along these lines until Olivo & Slavich in 1929 reported a large series of figures for the mitotic index of the developing heart in the chick.

Days development	Mitotic index	Intermitotic period in		% growth-rate calculated for 38 minutes
		hours	minutes	
2	22·5	19	42	—
3	21·2	20	55	—
5	16·9	26	11	15·7
7	15·0	29	24	9·4
9	8·3	53	11	10·2
11	10·0	44	3	4·8
13	7·0	70	15	5·7
15	4·4	100	33	4·0
17	3·7	117	39	2·5
19	2·8	155	55	4·2
21	1·2	363	7	1·8
10 (after hatching)	0·0	—	—	—

The fall in the mitotic index ran closely parallel with the fall in the percentage growth-rate of the organ, as determined by a special series of weighings. The time taken by one mitosis was calculated from these data by Olivo & Slavich: it turned out to be constant at 38 minutes. But the intermitotic period grew longer and longer, indicating that the later growth consists less than the former of proliferation and more of increase in size of the cells already formed.

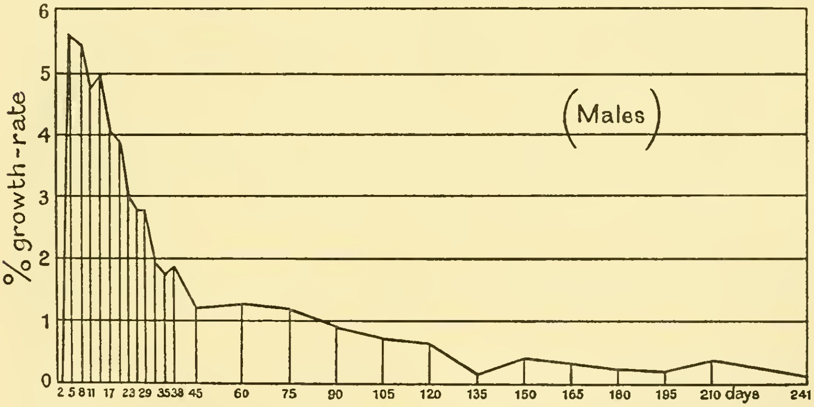


Fig. 43.

For a long time Minot's way of looking at embryonic growth in particular and growth in general was universally adopted, e.g. by Jenkinson, and even at the present time it is much used. But the Minot curve is undoubtedly based on a fallacy, and it was not long before a feeling that this was so began to arise. It was perhaps intensified by the appearance of such estimates as that of Mühlmann, who worked out the growth-rates in early stages of embryonic development in man as 3650 per cent. and above. It was not unnatural to enquire whether the Minot growth-rate of the original dividing egg-cell was even finite.

The dissatisfaction was voiced in 1917 by d'Arcy Thompson, who wrote as follows: "It was apparently from a feeling that the velocity of growth ought in some way to be equated with the mass of the growing structure that Minot introduced a curious and (as it seems to me) an unhappy method of representing growth in the form of what he called 'percentage-curves'. Now when we plot actual length against time we have a perfectly definite thing. When we differentiate this L/T we have dL/dT which is of course velocity, and from this by a second differentiation we obtain d^2L/dT^2, that is to say, the acceleration. But when you take percentages of y, you are determining dy/y and when you plot this against dx you have $\frac{dy/y}{dx}$ or $\frac{dy}{y.dx}$ or $\frac{1}{y}.\frac{dy}{dx}$, that is to say, you are multiplying the thing you wish to represent by another quantity which is itself continually varying, and the result is that you are dealing with something very much less easily grasped by the mind than the original factors. Minot is of course dealing with a perfectly legitimate function of x and y and his method is practically tantamount to plotting $\log y$ against x, that is to say, the logarithm of the increment against the time. [Cf. log. weight-age curves.] This could only be defended and justified if it led to some simple result, for instance if it gave us a straight line, or some other simpler curve than our usual curves of growth". This criticism was justified, for the Minot curve is certainly no simpler than the untouched growth curves; it merely falls instead of rising.

But d'Arcy Thompson did not point out the presence of a definite fallacy in Minot's way of looking at growth, a physiological rather than a mathematical one. This was grasped by Brody, who has written as follows: "Minot's method for computing growth-rates gives an exaggerated decline in the percentage rates of growth with increasing age simply because an arbitrary unit of time, e.g. a week, does not have the same significance at different ages. It is, for example, entirely appropriate to express the gain in weight during a week as a percentage of the weight at the beginning of the week (Minot's method) for a 6-month old chicken, because the weights (i.e. the number of cells or other reproducing units) at the beginning and end of the week are nearly the same as compared to the gain in weight. But to express the gain in weight during a week as a percentage of the weight of the body at the beginning of the week for a 7-day old chick embryo would be quite fallacious. It would correspond to expressing the gain in population in the U.S.A. from 1666 to 1927 as a percentage of the size of the population in 1666. The growth of the population of the U.S.A. in 1927 is proportional to the population in 1927 and not to the population in 1666. Similarly the number of cells produced in a 7-day old chick embryo should be functionally related to the number of reproducing cells (i.e. the weight) of the chick at 7 days of age and not to the number of cells at 1 day of age. In brief, growth is a continuous process and the rate of growth at every instant should be functionally related to the number of reproducing units at the given instant and not to the number of reproducing units which existed in some relatively remote past". In other words, Brody would prefer to ask not how much 100 gm. of embryo add on to themselves during the immediately succeeding period, but rather how many grams of those 100 gm. had been added on during a short preceding period. Murray's modification of Minot's method, in which the mid-increments instead of the daily increments are used as the basis for calculation, goes some way to meet Brody's criticism, for it enquires how much 100 gm. of embryo add on to themselves during half the preceding and half the following period, thus speaking in terms of a more instantaneous measure. Brody himself has made use of a similar amelioration. However, Brody's real point is that the fault lies in choosing an arbitrary length of time interval for all stages of development, in spite of the fact that they cannot possibly be equivalent for the embryo.

Brody might say that the embryo cannot be regarded as having been given an equal chance to accomplish its growth in each of the daily periods throughout its development. On the other hand, it might be argued that, though this is doubtless true as regards growth in weight, it is not true with respect to the activities of the embryo as a whole, which include many other processes, such as chemical differentiation. Taking the embryo as a whole in all its activities, the arbitrarily chosen and invariant period might be regarded as an adequate one. As we shall see later, this is essentially the same argument as that used by Murray against Robertson.

It may, however, be concluded that the Minot curve is only useful provided no theoretical conclusions are drawn from it, and that it is retained simply as a convenient method of comparing processes. Brody's own theories will be discussed later.

As against the theory which Minot built up from his experiments with growing animals, Murray has brought forward one convincing argument. Minot's theory of cytomorphosis involved the following propositions: (1) that the rate of growth depends on the degree of senescence, (2) that senescence is at its maximum when development begins, (3) that the rate of senescence decreases with age, and (4) that death results from the differentiation of cells. But, as Murray says, we have no real evidence to show us that the "degree of aliveness" at any given moment is in any way connected with the velocity of growth at that particular moment, or, more correctly, that the latter

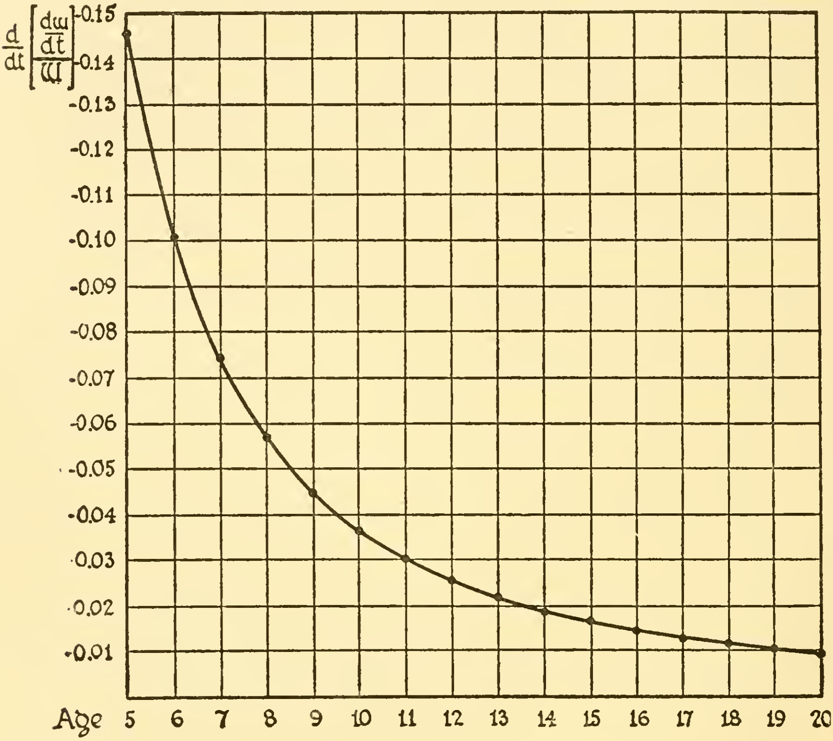


Fig. 44.

value is a true measure of the former. "There are other and perhaps more significant phenomena", said Murray, "than the growth rate, which change with age."

Murray himself proposed the use of a variant of the usual Minot curve by differentiating twice instead of once so as to get the acceleration and not the velocity. Thus, after having found the percentage growth-rate by the equation

$$\frac{d}{dt}\left[\frac{dw}{W}\right]=\frac{K}{t},$$

where $K = 3{\cdot}6$, he went on to find the negative acceleration for each day during embryonic growth:

$$\frac{d}{dt}\left[\frac{\frac{dw}{dt}}{W}\right] = -Kt^2.$$

This value, so obtained, is, as it were, the negative increment of the percentage growth-rate, and shows a regularly declining curve (for the chick) as in Fig. 44. Such a curve must obviously suffer from the same disadvantages as the curve from which it is derived, and does not escape from Brody's criticism that the arbitrarily chosen time-units are incommensurable at different developmental stages.

In 1922 Przibram observed that in many cases of post-embryonic growth the curve obtained by calculating according to Minot's method was extremely like a regular hyperbola, but he did not find that this was true for any example of embryonic growth. We shall see later what further use of this idea has been made.

2·6. Yolk Absorption-rate

Another way of regarding embryonic growth (of a lecithic egg) is to concentrate attention on the whole system, instead of upon the growth of the embryo alone. It was in this way that Gray treated the development of the trout embryo in the paper already mentioned (p. 369 and Appendix 1). He assumed that the rate of growth of the embryo was proportional to the dry weight of the embryo and to the dry weight of the remaining yolk. This idea had already been introduced for the trout by Kronfeld & Scheminzki (see p. 369 and Fig. 41) but Gray's figures were much more complete, and showed very clearly a falling off of growth towards the end of pre-natal life, when the yolk was becoming exhausted. Thus the wet weight of an embryo plotted against the time gave an **S**-shaped curve, which, however, was not symmetrical, for it had a point of inflection after about 70 per cent. instead of 50 per cent. of the development had been completed. This was of course well shown on the increment curve, which was skewly bell-shaped. Assuming that growth was proportional to the amount of yolk remaining as well as to the size of the embryo already formed, Gray developed an equation

$$x = y_0 - K_2 \log_e \frac{y_0 + K_2}{K_2}$$

(where x is the weight of the embryo at time t, y_0 the total yolk in the unfertilised egg, K_2 the amount of yolk combusted by one gram of embryo divided by the constant k in the equation

$$\frac{dx}{dt} = kxy,$$

x and y being weight of embryo and weight of yolk respectively) which he considered accounted very well for the observed facts. He deduced from it that there should be a period at the end of development when the wet weight of the larva (the whole system, embryo plus yolk) is decreasing, although the wet weight of the embryo itself is still increasing. During the major part of development the wet weight of both would increase, owing to the absorption of water from outside. From the equations the maximum weight of the larva should be reached when 0·86 gm. of yolk is still unconsumed, and in actual fact Gray found the peak at a point when 1·10 gm. was yet remaining.

Another possibility used by Gray to test his hypothesis was that as it was unlikely that the temperature coefficient of the growth process would be the same as that of the catabolism going on, there ought to be a measurable difference in the size of fishes raised at various temperatures at the end of their development. Experiments designed to reveal such differences gave the following results:

Temperature (°C.)	Mean weight of 100 embryos at the end of incubation (gm.)
15	13·35 ± 0·16
10	15·07 ± 0·18

so that the higher temperature not only accelerated the process, but, by accelerating the combustion more than the storage, led to a smaller finished fish. These results are curious, for it is generally understood that temperature changes alter the rate at which a growth-process goes on yet not the amount of end-product formed. The work of Barthélemy & Bonnet on the frog is an exact parallel to that of Gray on the trout, for these workers raised frog embryos at different temperatures with the following results:

Temperature (°C.)	Dry wt. of 300 eggs (gm.)	Dry wt. of 300 embryos (gm.)	$\frac{\text{Dry wt. of embryos}}{\text{Dry wt. of eggs}}$ (P.E.C.)
8	0·378	0·334	0·88
10	0·824	0·423	0·51
14	0·594	0·318	0·54
21	0·708	0·346	0·49

Temperature (°C.)	Dry wt. of 70 eggs (gm.)	Dry wt. of 70 embryos (gm.)	$\frac{\text{Dry wt. of embryos}}{\text{Dry wt. of eggs}}$ (P.E.C.)
8	0·092	0·079	0·85
10	0·128	0·101	0·79
14	0·097	0·082	0·84
21	0·107	0·088	0·83

If the second and third columns of this table are compared it will be seen that in the first series the French workers did get results like those of Gray, i.e. the higher the temperature the greater the combustions and the less the storage, but that in the second series there was no such effect to be observed. The Plastic Efficiency Coefficient (P.E.C.) is the most convenient way of expressing this relation (see Section 6·10). According to Gray it should change with temperature, for assuming his trout embryos to have the same percentage composition, no matter what the temperature, those raised at 15° would contain (each) 21·3 mgm. solid and consequently (since the eggs contain 43·4 mgm. solid) would have a P.E.C. of 0·50, while those raised at 10° would contain (each) 24·2 mgm. solid and consequently would have a P.E.C. of 0·56. I shall return to this subject in the section on general metabolism of the embryo; here it is only necessary to remark that the subject is clearly not yet settled and requires much more attention than it has so far received. At the same time, returning to the main theme, it must be remembered that Gray only made use of these temperature phenomena as one of the supports for his theory.

He drew another support from the fact that if his equations were correct, the product obtained by multiplying the dry weight of yolk by the dry weight of embryo should be at a maximum on the 71st day of development. This he found to be actually the case, as is shown in Fig. 45. And the growth-rate per gram of embryo (Minot growth-rate divided by 100?) was also proportional to the amount of yolk remaining.

Gray himself indicated a number of criticisms which might be brought against his views. Thus there is little *a priori* reason for supposing that the growth-rate of the embryo should be determined by the amount of yolk in the yolk-sac, for apart from anything else, the amount and nature of the syncytium in the yolk-sac wall might be a limiting factor. One would also expect that in the beginning when the yolk is very large compared to the embryo, the yolk would be present in excess, and would not exert any influence on growth-rate.

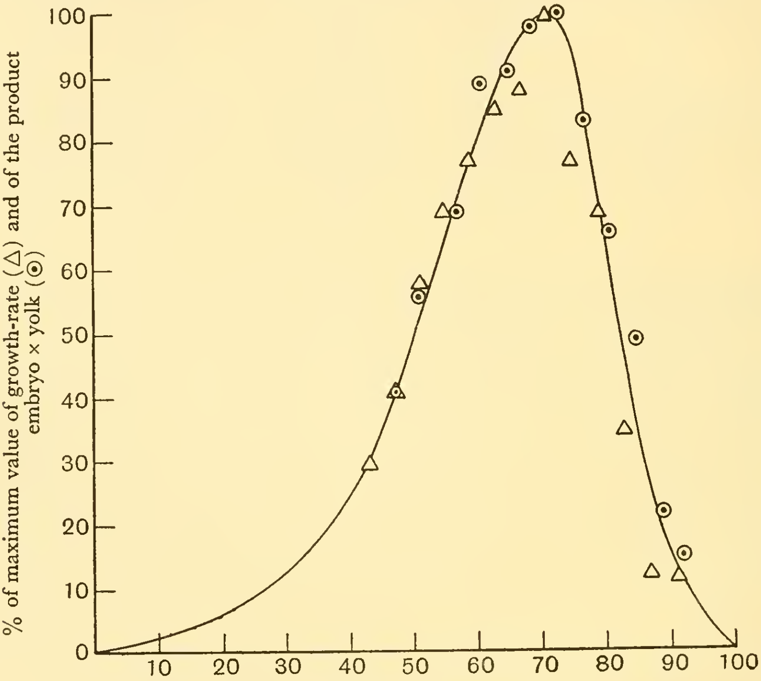


Fig. 45. Days of development.

2·7. The Autocatakinetic Formulae

The workers mentioned so far might be divided into three groups, those who have elaborated empirical formulae, those who have adopted the Minot point of view, and those who have treated the yolk plus embryo as one growth-system. Now a fourth and very large group consists of those who have been greatly impressed with the similarity which some empirical growth-curves show with the curve for a monomolecular autocatalytic reaction. This manner of looking at the subject is associated mainly with the name of Brailsford Robertson, who has in many papers and in a book specially devoted to the matter put it forward as the most fundamental approach to growth. Robertson was not, however, the first to notice the likeness. As early as 1899 it had been referred to by Errera, and Ostwald a little later. According to Monnier, Chodat of Geneva paid some attention to it in 1904. "One may regard growth, as M. Chodat has suggested", said Monnier, "as a complicated chemical reaction in which the living cell is the catalyst and the substances present are water, salts, and CO_2." Four years later (in 1908 on May 9) Ostwald's monograph on growth was published in the form of an inaugural dissertation, and only ten days later Robertson's first paper appeared in the *Archiv f. Entwicklungsmechanik.* Ostwald had treated the question in a rather unmathematical manner, but had fully explained the nature of his hypothesis; Robertson, on the other hand, gave the S-shaped curve a detailed mathematical treatment. "The carrying out of a progressive development in time has in animals a single characteristic type; the rapidity of the process begins at a low value, increases with the continuance of the action and falls off again at the end, in other words the type of curve is S-shaped." This was as far as Ostwald went, but he did not fail to point out that the S-shaped curve was identical with that of an autocatalytic or an autocatakinetic reaction. An important point to note is that Ostwald's curves were all curves of absolute weight—he did not in any instance plot increment curves.

Robertson began by an explanation of the mathematical properties of the autocatalytic curve. The differential equation characteristic of the initial stages of an autocatalytic monomolecular reaction is as follows:

$$\frac{dx}{dt} = k_1 x \, (a - x),$$

which expresses in mathematical symbols the fact that the velocity of the transformation is, at any instant, proportional to the amount of material which is undergoing change and to the amount of material which has already undergone change. If, however, the reaction has proceeded so far that the depressant effect of the products is measurable, then the previous equation becomes

$$\frac{dx}{dt} = k_1 x\,(a - x) - k_2 x^2.$$

Now when the reaction has proceeded half-way to equilibrium, i.e. when $x = \frac{1}{2}a$, the equation becomes

$$\log \frac{x}{A - x} = Ak\,(t - t_1),$$

or

$$\log \frac{x}{A - x} = K\,(t - t_1),$$

and this is the well-known equation for the **S**-shaped curve, where x represents the body-weight (not the increment) at time t, A represents the maximum of final weight which the organism is to reach, t_1 the time at which half this maximum body-weight has been attained, and K a constant which has to be determined from a known value of x at a given time t. By differentiating, it may be found that dx/dt is at a maximum when $x = \frac{1}{2}A$—in other words, that the rate of increase in weight is greatest when half the autocatalytic curve has been passed through. Robertson proceeded to apply his calculation to the growth of white rats, figures for which had been reported by Donaldson, Dunn, & Watson, with excellent results over part of the curve, though not when the age amounted to more than 100 days. At the time it seemed as if this was a most convincing example of the value of the autocatalytic theory, but maturer consideration showed that this 100 days was but a third of the possible life-span, and the comparison of the two curves as made by Lotka, for instance, does not look so impressive.

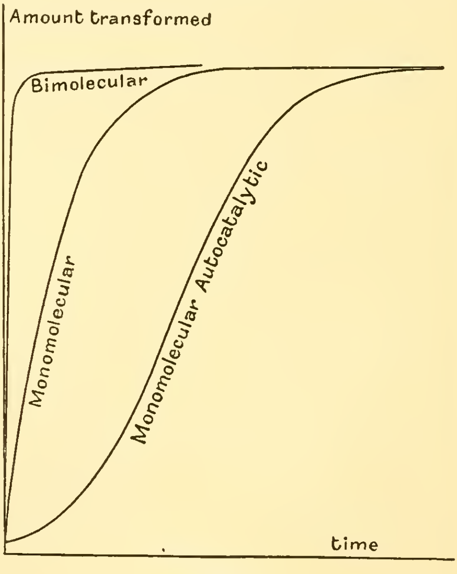


Fig. 46.

Fig. 46, taken from Robertson's first paper, illustrates the relation between the curves for the three chemical reactions, while in Fig. 47 is seen the theoretical and the experimental curve compared. In this same first paper, Robertson applied his autocatalytic equation to the growth of man, frog, a vine, and to certain organs. The frog figures, which were those of Davenport, were the nearest approach to embryonic growth dealt with by Robertson, and they are given in Table 54. They showed a good measure of agreement, but whether it was right to say, as Robertson did in his summary, that "in all probability cell growth or the synthesis of cytoplasm is an autocatalytic reaction" is a question that subsequent workers have not by any means answered with a bald affirmative.

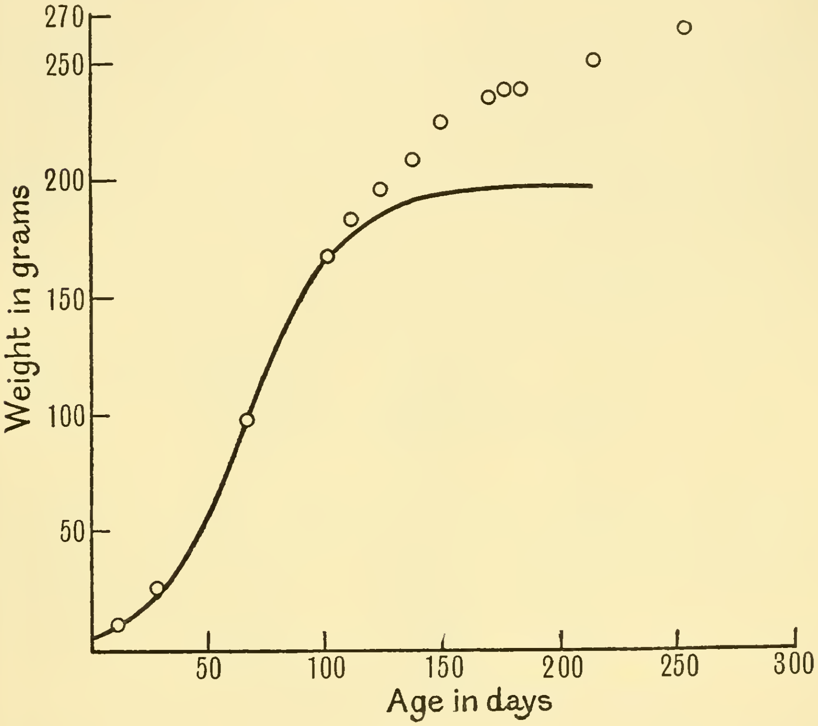


Fig. 47.

Robertson pointed out that his new interpretation of growth curves fitted in very well with the views that had already been advanced by Loeb. Loeb had suggested that the processes of cell-division and growth were simply expressions of a more or less rapid return to the chemical equilibrium between nucleus and cytoplasm which had been temporarily shifted through the process of fertilisation. He thought that there occurred in the sea-urchin's egg during the early stages of its development a great synthesis of nuclear material, and that this synthesis progressed, roughly speaking, all the more rapidly the more nuclein was formed. The velocity of nuclear synthesis, said Loeb, increases with lapse of time in geometrical progression. Further, various observers, using the Q_{10} formula, had concluded that the growth-process had a "chemical temperature coefficient", so Robertson felt fully justified in speaking of a "master-reaction" of growth, and in thinking of it as autocatalytic—a reaction, which, because it would be slower than any other, would act as the limiting factor of growth, and would impress its own particular character on the general appearance of the whole process from outside.

Table 54. *Larval growth of frog (tadpole).*

Days after hatching	Davenport's experimental figures (mgm.)	Robertson's figures calculated from autocatalytic formula (mgm.)
1	1·83	1·64
2	2·00	2·03
3	—	—
4	—	—
5	3·43	3·90
6	—	—
7	5·05	6·00
8	—	—
9	10·40	9·01
14	23·52	23·46
41	101·00	110·90
81	1989·90	112·00*

* "Obviously another growth-cycle supervening", said Robertson

In his later writings, Robertson added further demonstrations of the applicability of the autocatalysis equation to growth, but he also added a large amount of extremely speculative considerations, such as the "nutrient level", the "endogenous catalyser", etc., into which we cannot here enter. Some of his suggestions, for example that the autocatalyst is lecithin, may be regarded as now definitely out of court. He showed, however, that the pre-natal growth in man (using Zangemeister's figures) was susceptible of description in an autocatalytic curve with its early "autokinetic" phase (Robertson's terminology), and its late "autostatic" phase. He also used, for the last part of the curve, data on the weight of new-born infants, born at different times, some earlier and some later than the normal. We have, moreover, already seen that the curve constructed by Vignes for human embryonic growth shows an S-shaped conformation.

In 1926 Robertson published a long paper in which he reviewed the work which he and his collaborators had done on the subject of the autocatalytic theory of growth. Here he showed that the fall in the relative value of the velocity constant of (asymmetric) autocatalysis during the embryonic growth of the mouse followed almost exactly the same curve as the fall in the chemical nucleoplasmatic ratio as determined by LeBreton & Schaeffer (see Section 10·2). It is not clear, however, what significance is to be attached to this finding, especially as Crozier and Brown have shown that at least two velocity constants must be postulated in a given cycle. Robertson himself drew no theoretical conclusion from it.

Robertson regards the several growth-cycles distinguishable in the life of an animal as being independent, in that they each have a different catalyst. The first of these in the case of the mouse has an equation of the type

$$\log \frac{x + B}{A - x} = k\,(t - t_1),$$

where x is the growth attained at time t, A the maximum growth attainable in the cycle under consideration, t the time required to attain half the maximum growth and k a specific velocity constant. B is another specific constant, an index of the asymmetry of the curve. The second and third cycles have the formula

$$\log \frac{x}{A - x} = k\,(t - t_1),$$

where the symbols are as before. He considers that skewness or asymmetry originates probably in a progressive diminution of the velocity constant as described above.

Robertson's suggestions were not allowed to go uncriticised. Meyer, in his report of data for the growth of the human foetus, took occasion to attack both them and the traditional Minot standpoint. On the whole, Robertson's autocatalysis theory emerged with less damage than Minot's cytomorphosis theory. Meyer brought into the light what had been one of the most disturbing features of the Minot method, namely, the absurd values which the percentage growth-rate has in the earliest stages, e.g. five hundred and forty billion per cent. But, as Meyer seems to have completely failed to understand that Robertson and Minot were using different methods in calculating the "growth-rate", his criticism of Robertson was not of any importance. On the other hand, he did draw attention to the fact that growth-curves can be very misleading if it is not remembered that, though in the early stages the absolute growth is minute, the relative growth is enormous. "The weight of the impregnated human ovum", said Meyer, "is approximately 0·005 mgm., and yet investigators in all seriousness indicate its weight on a short ordinate reading in grams or even hundreds of grams. Little wonder, then, perhaps, that Robertson, Ostwald, and Read have unwittingly assumed that the curve of growth in man and mammals hugs the abscissa for several months as the curve of autocatalysis does." This was a good tilt at a common fallacy, but Meyer did not point out that it could be remedied by using log. paper, and he left it quite open to Robertson to reply that, even when strictly comparable quantities were taken, the **S**-shaped curve or a succession of **S**-shaped curves still resulted.

More serious criticisms than these have, however, been brought against the Robertson method of treating embryonic growth. Luyet has pointed out that it may suffer more than the other methods from illusory differences in material. Again, Murray has written as follows: "(1) The formula demands the introduction of three separate constants which must be separately determined for every set of figures collected. (2) The equation does not give the weight as a function of age throughout life but only during an arbitrarily selected part of the growth-cycle. For these two reasons the equation as a practical simplification is not of great value. If the equation were in such a form that knowing the species, the age, the $T°$, and other environmental variables, one might calculate the weight and growth-rate, it might be of use. But as it stands now, it is necessary in each case to collect complete statistics and then find a mathematical expression of the figures obtained. For instance, all three constants in the equation for the growth of South Australian males differ from the constants used in the equation for the South Australian females. As one cannot extrapolate, the formula, like the man with one talent, returns what it receives. In fact as it covers only a section of the growth-curve, it yields less information than the original data. Moreover, as a rational account this description of the synthetic processes of growth is misleading, since (3) by this theory the growth-rate is proportional to the increment gain in weight regardless of the weight of the organism, or in other words, disregarding the amount or concentration of the reacting substances. (4) Figures for the growth of colonies as well as of individual organisms are said to be described by autocatalytic equations and are classed together. When growth is expressed in terms of percentage increase in mass, however, the important distinction between phylogenetic and ontogenetic growth is made evident; for the former, after a short latent period, in the presence of an experimentally modified environment proceeds at a constant rate [cf. Richards], while the latter does not. The S-shaped curve is the result of a limited and unrefreshed culture medium. The individual organism, however, instead of maintaining a constant growth-rate shows from the beginning considerable negative acceleration. (5) The S-shaped curve is not specific, for there are some physico-chemical processes not considered to be autocatalytic which are described by a similar curve. Finally, and this is the main objection, (6) chemical differentiation is not taken into account by the autocatalytic theory, which is based on the conception that there is some one master monomolecular reaction, which, being the slowest of the chain of reactions concerned in the phenomenon of growth, determines the velocity of the entire process. As there is no direct way of measuring the product of the master-reaction, the increase in the body-weight of the whole embryo is taken to represent the product. In view of the marked changes in chemical constitution which take place in the tissues with age there is no reason to suppose, and in fact it is extremely unlikely, that the total weight can be taken as an index of the amount or concentration of any one chemical substance". The tendency that has existed in the past to take the simple weight of the embryo as the sign *par excellence* of its developmental stage and its "aliveness" is only another case of the reluctance to judge by "ensemble", as Broca called it, instead of by single indices, which was so long the bane of physical anthropology. In this case, it is the increasing application of chemical methods to the embryo which has shown the superficiality of regarding mere weight as the pre-eminent factor. Lotka has drawn attention to this point of view, and is inclined to compare embryonic growth to the growth of a population such as Pearl & Parker have studied in *Drosophila*. Janisch treats the **S**-shaped growth-curve as the reciprocal of a catenary exponential curve. These presentations have the advantage that they do not prejudge the issue from a physicochemical angle.

Exception to Robertson's views has been taken on quite other grounds by Snell, who points out that Robertson's equation

$$\frac{dx}{dt} = K_1 Ax - K_2 x^2$$

and Crozier's modification of it

$$\frac{dx}{dt} = (K_1 + K_2 x)\,(A - x)$$

(where x is the concentration of the end-product at time t, A the concentration of the substrate at time t, and K_1 and K_2 the velocity constants of the forward and reverse reactions respectively) do not take into account the fact that the system concerned is not a closed one. All the time the embryo is growing it is also eating, i.e. absorbing nutritive material, and in addition it is giving out waste products. Accordingly these equations derived from the law of mass action as we know it in the inorganic world do not allow for the effect of increasing size on the concentration of the reagents involved in growth. The equations hold true only on the condition that the volume occupied by the resulting substances remains constant, and since a growing organism is constantly increasing in volume, this condition is not met. "When a chemical process is carried out in the laboratory," said Snell, "the reagents are ordinarily dissolved in water or some similar solvent, and the volume of the solvent is kept constant throughout the whole process. To make the conditions of a laboratory process comparable to those involved in the synthesis of new protoplasm the volume of the solvent would have to be increased as fast as the amount of the end-products is increased. As the solids of new protoplasm are formed, they do not stay in the same little parcel of liquid occupied by the old, rather they cause the liquids to expand with them. Hence the volume occupied by the end-products of growth is proportional to their amount, and the concentration of these products, instead of increasing, remains constant. This is a very important difference, for it is on the concentration and not the amount of reagents that reaction velocity depends." Thus the equations of Robertson and

Crozier would only be true if the chick embryo, for instance, began as a kind of watery ghost of dimensions equivalent to those it normally has at hatching, and if development consisted in the gradual accumulation of solid substances within it. This singular kind of preformation certainly does not exist in reality. Snell developed another equation,

$$\frac{dx}{dt} = \left(K_1 + K_2 \frac{x}{cx + V_0}\right)(A - x)$$

(where A is the initial amount of substrate, x the amount of end-product at time t, cx the corresponding concentration at time t, V_0 the volume of the organism at the beginning of the growth-cycle, and K_1 and K_2 the velocity constants as before), which he regards as the correct form for the representation of an autocatalysed monomolecular reaction in which the conditions are similar to those in a growing animal, or in other words, where the volume occupied by the reagents increases in proportion as the end-product increases. Snell then showed that the curves obtained by this equation did not resemble any of the empirical growth-curves in the literature, and therefore concluded that there is no sound basis for assuming that the master reaction is either monomolecular or autocatalytic. Thus in the case of embryonic growth, where $\frac{x}{cx + V_0}$ is almost constant, the curve approximates to that of a non-autocatalysed monomolecular reaction, and again to nothing that is given by any actual embryo.

In any case the only instance where a sigmoid curve has been shown to fit the growth of an individual foetal organ is in the work of Fauré-Fremiet & Dragoiu on the lung of the embryo sheep.

Robertson's point of view was adopted in the earlier work of Brody and his collaborators, who, however, introduced new viewpoints into it. Instead of plotting the absolute weights of the embryo against time, they plotted the increments per time-unit, thus obtaining curves similar to those in Fig. 34 above. Obviously, in a case where the absolute growth-curve was S-shaped, these increment curves would be doubly S-shaped, rising to a maximum at half-time and thereafter falling away. Or, put mathematically, from the differential equation

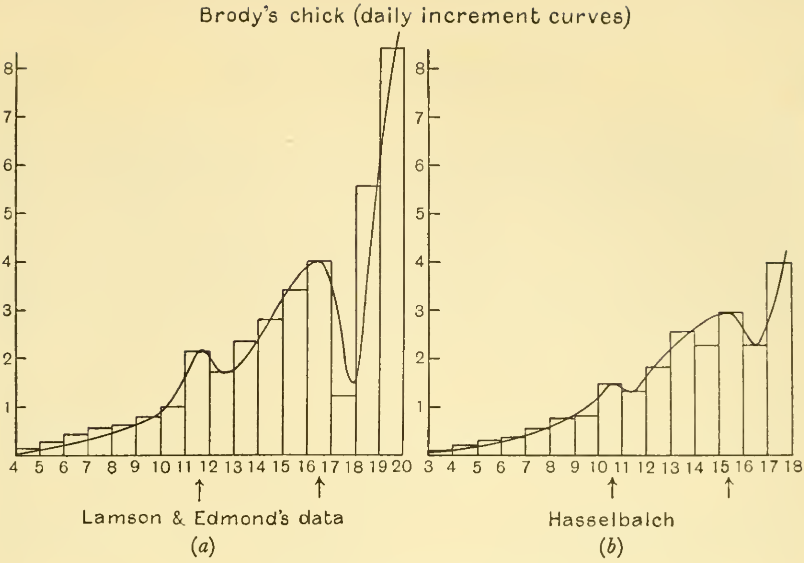
$$\frac{dx}{dt} = Kx\,(A - x)$$

it follows that the velocity of change in an autocatakinetic system progressively rises from zero hour to a maximum value when $x = \frac{1}{2}A$ and afterwards constantly falls. When the data are plotted in this way the existence of growth-cycles, whether real or not, comes out much more clearly than when the absolute curves alone are used. It is often said, however, that the increment curve emphasises small fortuitous variations more than the absolute curve, and certainly there are cases, notably the chick embryo itself, where the increment curve shows up cycles which are but poorly shown on the absolute weight curve. Brody & Ragsdale in their first memoir on this subject dealt only with the growth of the cow, and concluded that one complete growth-cycle was accomplished in the foetal condition. A later paper considered the growth of the fowl, for which, on the data of Card & Kirkpatrick for growth after hatching, two cycles appeared, with maxima at 9 and 18 weeks respectively. For the growth of the embryo, the data of Hasselbalch and of Lamson & Edmond, re-arranged by Brody, gave two maxima also, but at slightly different times, thus:

Lamson & Edmond	11·5 and 16·5 days
Hasselbalch	10·5 and 15·0 days.

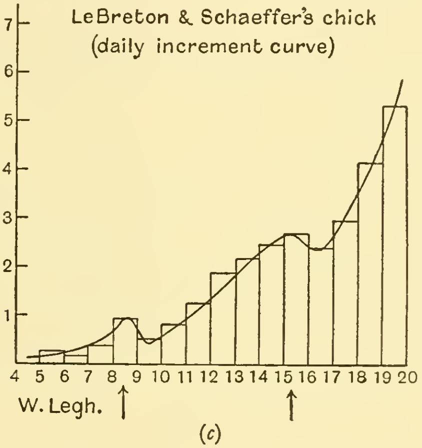
Brody's figures for these curves are shown in Fig. 48. LeBreton & Schaeffer, who subsequently published a series of chick embryo weights, found maxima at 9 and 15 days, but in Murray's series there is no peak at any time, except a doubtful one on the 16th day, and a plateau between the 12th and 15th days. Schmalhausen's data again, when the increments are calculated, show in the case of both series peaks at 10 and 12·5 days, with an additional one, in the case of his 1927 series, at 17 days.

In the face of this consensus of evidence, it is not altogether easy to conclude with Murray that "Brody's rhythmic growth curves were due to chance variations". It is true that Lamson & Edmond; Hasselbalch; and LeBreton & Schaeffer used too few embryos in their work, but even in Murray's own work, where about 650 embryos were used, there is an unexplained drop between the 17th and 18th days, as well as a plateau between the 12th and 16th days, both quite outside the probable error. Murray did not, it is true, get the 18th-day drop in all his experiments. In Schmalhausen's two series about 400 embryos were used.

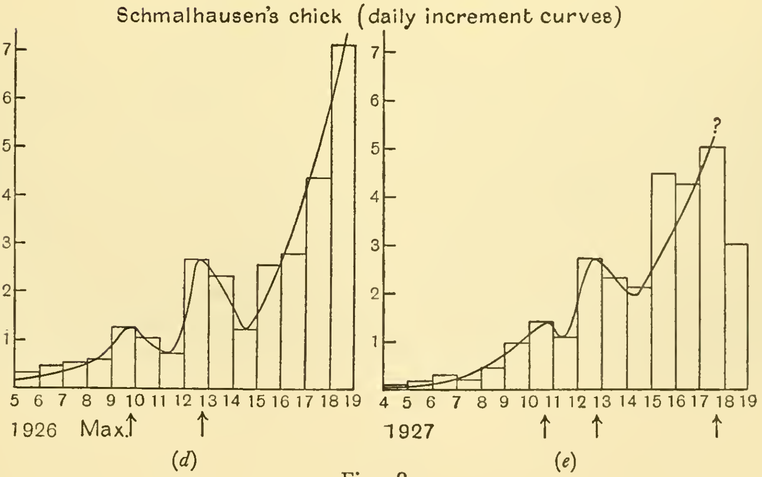


Lamson & Edmond's data
(a)

Hasselbalch
(b)



(c)



(d)

(e)

Fig. 48.

It may further be argued that weighing is a very simple process, and it is, therefore, difficult to see why errors should arise which should reflect themselves in these rhythmic curves. A greater degree of scepticism would be justified if they were the results of a complicated estimation method for a chemical substance. But, as it is, these curves form perhaps the best evidence which at present exists for the applicability of the Ostwald-Robertson view to embryonic growth. In the absence of a really exhaustive statistical investigation of the growth of the chick in the egg, these rhythmic curves must be accepted for what they are worth. They would be more convincing if all the workers had found peaks in the same places, but the variation which exists is no argument against the reality of the phenomenon in view of the fact that different breeds of hen were used. Further work is greatly needed to clear up this question. If the peaks on the daily increment curve do turn out to be real, it may be possible to relate them to the peaks of normal mortality which Payne and others have studied, and which will receive further consideration later. (See Fig. 443, Section 18·2.)

The autocatalytic curve has also been found by Robertson to fit the data of Stotsenberg already referred to for the growth of albino rat embryos, and a peaked curve is obtained when the daily increments are plotted against time. But, as will be seen, Brody's exponential formula also fits these data, and it is probably right to conclude, as McDowell and his collaborators do, that the figures are not sufficiently good to allow us to distinguish between the two formulae. They cannot be regarded as supporting, therefore, any particular theory of embryonic growth.

2·8. Instantaneous Percentage Growth-rate

Brody introduced still another way of representing the facts. He defined the "genetic growth constants" of animals as being the same within each genetically identical group of animals, and as corresponding to specific velocity constants and equilibrium constants in chemical actions *in vitro*. It may be seen from Fig. 49 taken from Brody's paper that the mature weight of the animal in its life-span, *A*, is approached by successively decreasing gains in weight after the point of inflection of the sigmoid curve has been passed. The velocity of growth, therefore, declines in a geometrical progression with age. The normal animal reaches, as Brody puts it, under a given set of favourable conditions (much more constant, of course, in egg or uterus than outside), a mature weight which is characteristic of its own species, just as the product of a chemical reaction *in vitro* reaches under a given set of conditions a definite equilibrium concentration characteristic of its kind. The mature weight A was determined by Brody for a large range of animals

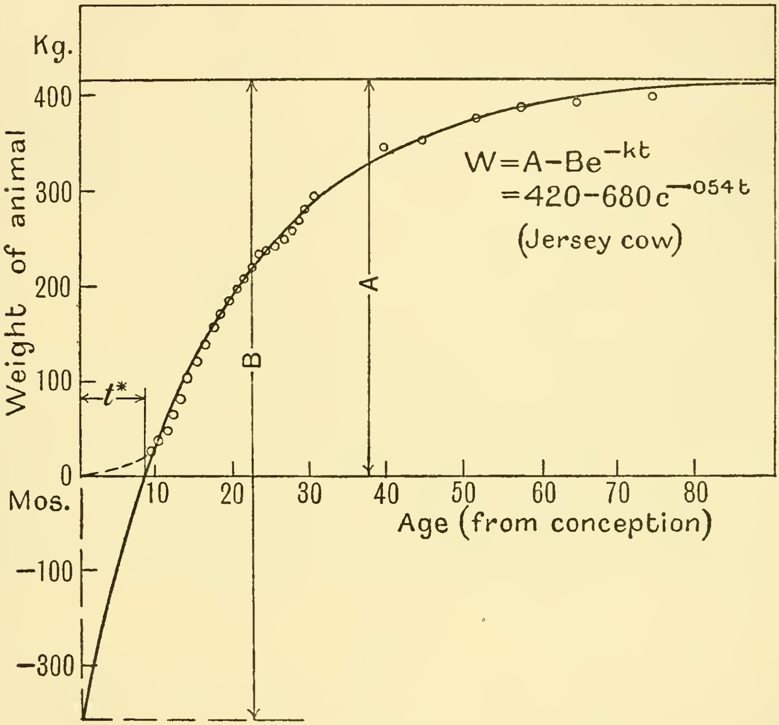


Fig. 49.

by a graphical method. Now, in this process of geometrical progression in which the increments in unit time are becoming progressively smaller, it is found that in each unit of time the gain made in percentage of the gain made in the previous unit of time is a constant. Thus, in the autostatic growth-phase of the rabbit, for example, the gain is, during each month, 78 per cent. of what it was during the previous month. Brody calculated out this constant, k (simple growth persistency), for a great many animals. It corresponds to the specific velocity constant in chemical equations.

Finally, B is the difference between the mature weight of the animal and the weight the animal would have had at conception (a minus quantity) if the whole of growth was representable by the curve for the autostatic or self-inhibiting phase. This genetic growth-constant also was found for many animals by Brody. From Fig. 50 it can be seen that, the higher the value of k, the more rapidly the mature value is approached (pigeon > mouse > rat > guinea-pig > sheep > pig > cow > man), and that the fact can be equally well accounted for on the assumption that a substance is used up during growth, or on the assumption—perhaps more likely in view of the work of Carrel and his collaborators on tissue culture (reviewed by Pearl)—that during growth a growth-retarding substance is produced according to the law of monomolecular change. The paper of Brody, Sparrow & Kibler was concerned with age equivalence. They showed that, with the aid of the formula previously established by Brody,

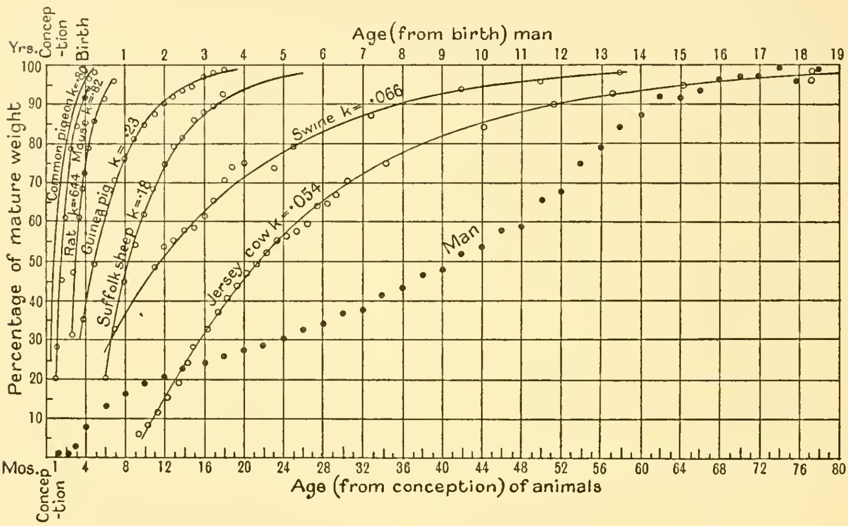


Fig. 50.

$$W = A - Be^{-kt}$$

(where W is the weight at age t, A a genetic growth-constant, the mature weight, B another genetic growth-constant which increases in value with increase in length of the processes preceding the point of inflection, and *k* a third genetic growth-constant, the fractional decline in the velocity of growth), it was possible to plot growth-curves for all animals to the same base, and so to determine their age-equivalence. Thus they found that 1 rat month was equivalent to 11·91 cow months, and 1 guinea-pig gram to 509·1 cow grams. They finally constructed a table (Fig. 51), in which the value of *k* was given for a great many animals, and a logarithmic graph, from which can be read off the time in months (conception age) at which the animal with the constant *k* in question will arrive at 10, 20, 30 or 90 per cent. of its mature weight.

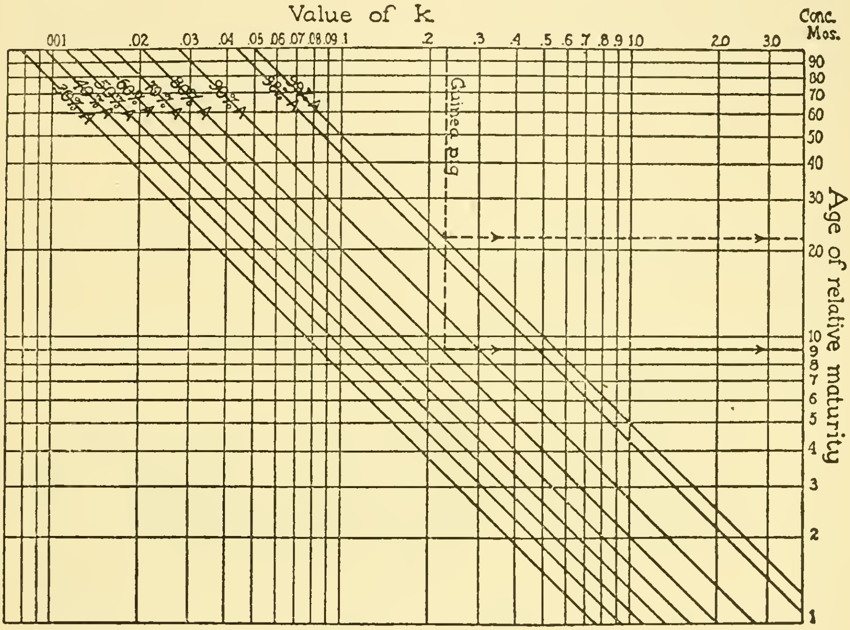


Fig. 51.

Brody next considered the growth-constants during the autokinetic or self-accelerating phase of growth. He subjected the methods which had previously been used to represent growth to severe criticism, part of which has already been referred to. Thus, in the case of Minot's method, increments of growth are regarded as being added on discontinuously at the terminal points of arbitrary time periods, whereas growth is really a continuous process, and the mathematical expression for it must take account of its smooth nature. Brody found the relationship between the relative rates of growth k

$$\left(\frac{\frac{dW}{dt}}{W} = k\right)$$

to be

$$k = \log (R + 1),$$

where R is Minot's percentage growth-rate. The latter can, therefore, only be used when it does not exceed 10 per cent. for the period under consideration, i.e. never in embryological work.

Brody also criticised the methods of Pearl, whose equation

$$\frac{dW}{dt} = \frac{k}{t - a}$$

does not take account of the fact that part of growth is self-accelerating, and also Pearl & Reed's modification of the original Robertson equation. He maintained that the best way was to plot the log. weight against the age, when, if the result is a straight line, the rate of instantaneous growth, k, must be a constant. This will not of course be confused with the fractional persistency constant referred to above, for the latter only refers to the autostatic or self-inhibitory phase. The autokinetic or self-acceleratory phase is clearly the more important and interesting for embryologists.

Figs. 52 *a*, *b*, taken from Brody's paper, show the data of various workers for the wet weight of chick embryos treated in the way described, namely, the log. weight plotted against the age. It will be noted that a series of straight lines result, forming a system concave to the abscissa and rising rapidly but more quickly at first than later. The curve is thus the exact opposite of the Minot

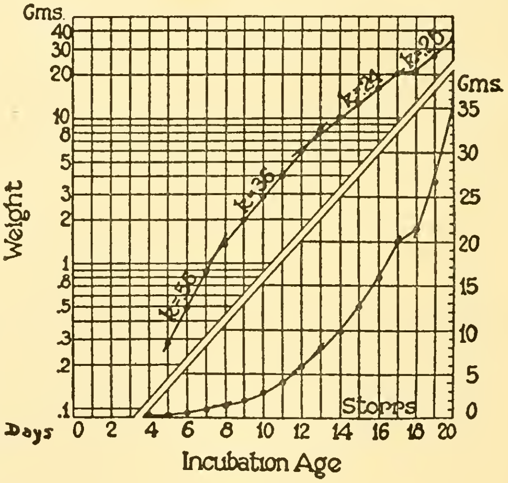


Fig. 52 *a*.

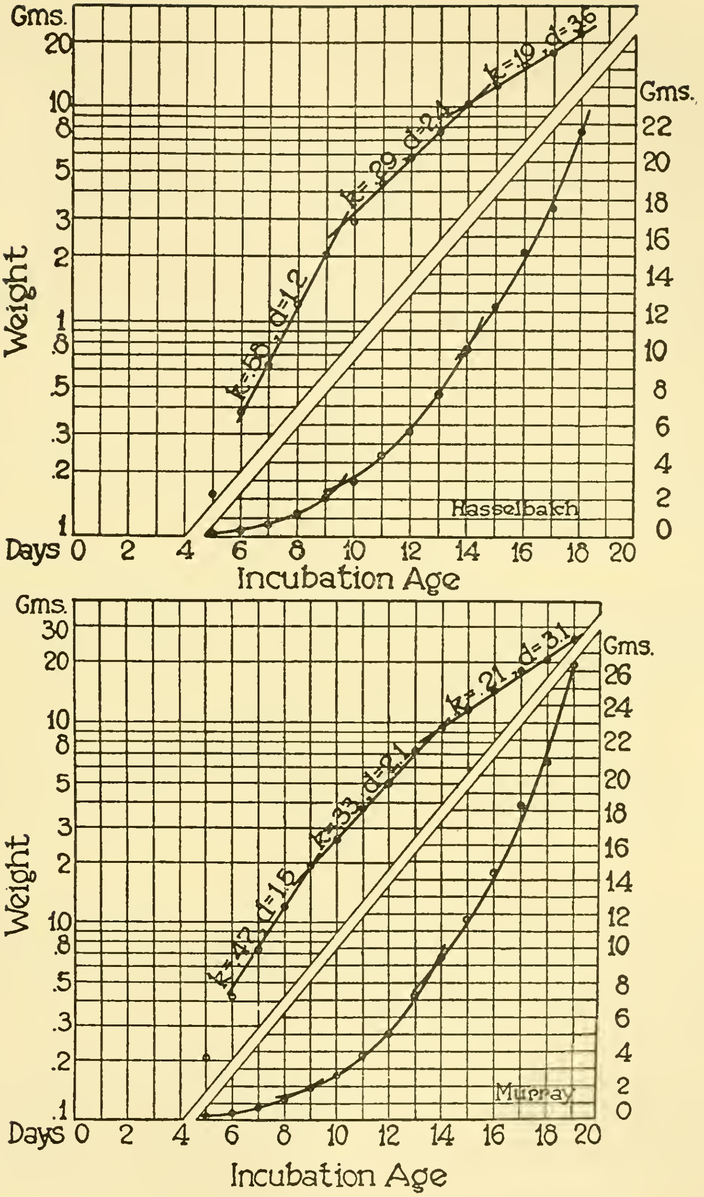

Gms.
20
10
8
5
3
2
1
.8
.5
.3
.2
1
Weight
k=.56, d=1.2
k=.29, d=2.4
k=.19, d=3.6
Gms.
22
20
18
16
14
12
10
8
6
4
2
0
Hasselbalch
Days 0 2 4 6 8 10 12 14 16 18 20
Incubation Age
Gms.
30
20
10
8
5
3
2
1.0
.8
.5
.3
.2
.1
Weight
k=.42, d=1.5
k=.33, d=2.1
k=.21, d=3.1
Gms.
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Murray
Days 0 2 4 6 8 10 12 14 16 18 20
Incubation Age

Fig. 52 *b*.

curve, which falls so markedly during the same time. Thus the growth-rate expressed in this manner also falls off as time goes on, or rather rises less and less rapidly, becoming eventually asymptotic to the mature value.

This curve is undoubtedly a great improvement on Minot's, for it involves no arbitrary time period and depends on the differential calculus, which has as its special province the evaluation of instantaneous change. An infinitesimally small period dt, and an infinitesimally small increase of weight dW, are the basis of its operation. Then all the infinitesimal differences can be added together (i.e. integrated). Thus:

$$\frac{\frac{dW}{dt}}{W} = k$$

becomes, if the number of dt's and dW's is infinitely large (n), when integrated, $W = Ae^{kt}$, for

$$\left(1 + \frac{k}{n}\right)^{nt} = e^{kt},$$

A being the weight at the beginning of the whole period. If this is turned into logarithms

$$\log W = \log A + kt,$$

or

$$k = \frac{\log W - \log A}{t},$$

and, as where growth is being considered A is, to all intents and purposes, 0,

$$k = \frac{\log W}{t}.$$

The instantaneous relative growth-rate for a unit time is the sum of all the instantaneous rates during the given unit of time, and may therefore be multiplied or divided, according to the time-unit in which it is desired to express it.

The log. weight/age graph is, therefore, a measure of the instantaneous growth-rate, and the value of the constant k which can be calculated from the last equation will give the slope of the straight, or approximately straight, line. The log. weight/age graph could, of course, have been plotted by Minot, but Brody's use of the differential calculus was required to show that the slope of the curve gave an instantaneous growth-constant. Thus, in the example given, Lamson & Edmond's data, the constant is 56 from the 5th to the 8th day of development (very steep slope), 36 from the 8th to the 13th day (less steep slope), 24 from the 13th to the 18th day (still less steep), and 25 from then onwards. The higher numerically the constant k, the steeper the slope, and consequently the greater the instantaneous growth-rate. In Figs. 53 *a*, *b* and Table 55 are shown most of the weights and processes in the hen's egg whose constants have been calculated by Brody. Each system grows at a rate peculiar to itself.

Murray, as we have seen, also plotted log. weight against age, but he did not get a straight-line relationship; on the contrary, the resulting curve was concave to the age (abscissa). McDowell, again, got a similar concave curve for the pre-natal growth of the mouse, and there is much point in his criticism of Brody's work: "Brody draws a series of straight lines through corresponding exponential curves and concludes that growth-rate does not decline continuously but by abrupt drops between periods of uniform rate. Since any curve can be approximated by a series of straight lines, the critical significance, both of the specific number of straight lines, and of his general conclusions, seems somewhat questionable"*.

Table 55 includes also a column in which the time taken for the embryo or a corresponding entity to double its weight or amount is shown. For, when the instantaneous percentage growth-rate is constant, the time intervals between doubling of weights are constant; therefore, from the expression

$$W = Ae^{kt},$$

at a certain time

$$\frac{\log 2}{k} = \frac{0{\cdot}695}{k},$$

and, as k is found to be for the rat embryo 0·53 or 53 per cent., the time required for it to double its weight must be $\frac{0{\cdot}695}{0{\cdot}53}$ or 1·3 days. Further, if growth in weight can be taken as a measure of the increase in the population of cells in the body, a new cell-generation is produced every 1·3 days on an average, and the cell-division frequency is 1/1·3, i.e. 0·77 times per day. It is thus possible to determine, as Brody says, the mean life of a mother cell before it divides into two daughter cells.

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* Nevertheless, McDowell himself admits a discontinuity between pre-axial and axial growth, as we have seen on pp. 394 and 396.

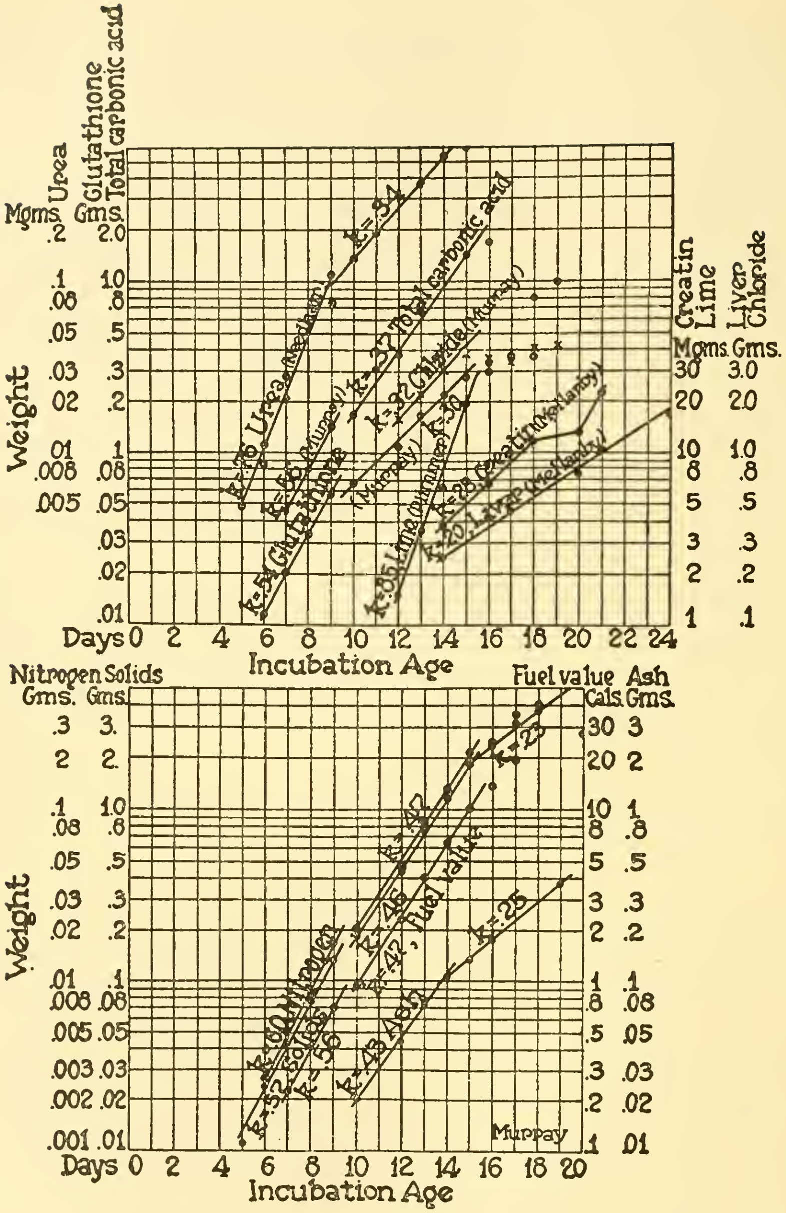
Mgms. Urea
Gms. Glutathione Total carbonic acid
.2 2.0
.1 1.0
.08 .8
.05 .5
.03 .3
.02 .2
.01 .1
.008 .08
.005 .05
.03
.02
.01
Weight
Days 0 2 4 6 8 10 12 14 16 18 20 22 24
Incubation Age
Creatin Lime
Liver Chloride
Mgms. Gms.
30 3.0
20 2.0
10 1.0
8 .8
5 .5
3 .3
2 .2
1 .1
K=.76 Urea (Needham)
K=.84
K=.37 Total carbonic acid
K=.60 (Murray)
K=.54 Glutathione
K=.32 Chloride (Murray)
(Murray)
K=.30
K=.85 Lime (Dunnmore)
K=.28 Creatin (Mellanby)
K=.20 Liver (Mellanby)
Nitrogen Gms.
Solids Gms.
.3 3.
2 2.
.1 1.0
.08 .8
.05 .5
.03 .3
.02 .2
.01 .1
.008 .08
.005 .05
.003 .03
.002 .02
.001 .01
Weight
Days 0 2 4 6 8 10 12 14 16 18 20
Incubation Age
Fuel value Cals.
Ash Gms.
30 3
20 2
10 1
8 .8
5 .5
3 .3
2 .2
1 .1
.8 .08
.5 .05
.3 .03
.2 .02
.1 .01
K=.23
K=.47
K=.46
K=.42, Fuel value
K=.25
K=.60 Nitrogen
K=.52 Solids
K=.56
K=.48 Ash
Murray

Fig. 53 *a*.

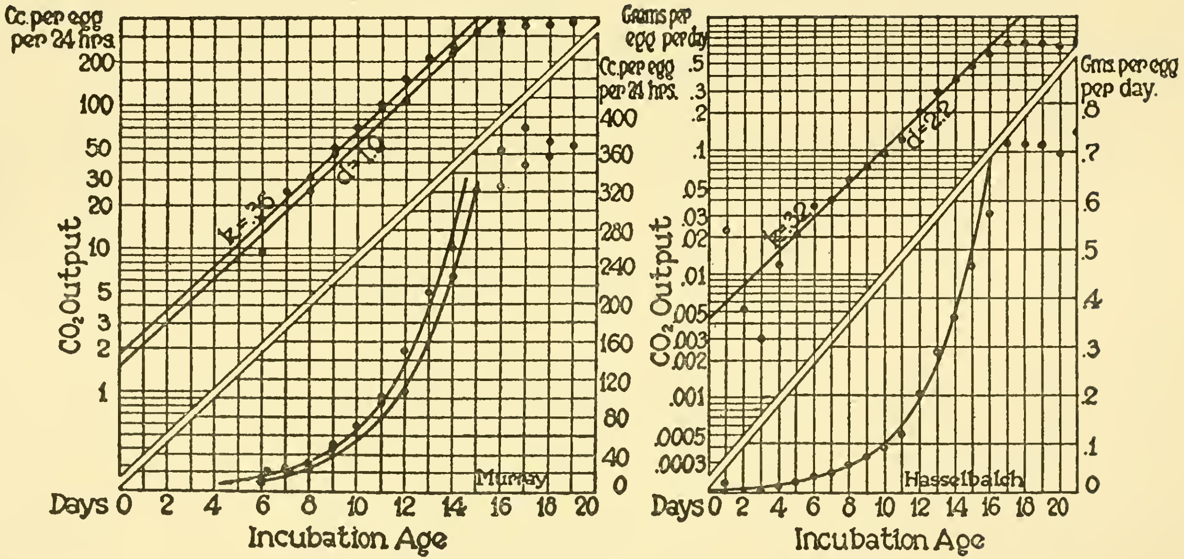
Cc. per egg per 24 hrs.
200
100
50
30
20
10
5
3
2
1
CO₂ Output
Days 0 2 4 6 8 10 12 14 16 18 20
Incubation Age
k=.36
d=1.9
Murray
Cc. per egg per 24 hrs.
400
360
320
280
240
200
160
120
80
40
0
Grams per egg per day
.5
.3
.2
.1
.05
.03
.02
.01
.005
.003
.002
.001
.0005
.0003
CO₂ Output
Days 0 2 4 6 8 10 12 14 16 18 20
Incubation Age
k=.32
d=2.2
Hasselbalch
Gms. per egg per day.
.8
.7
.6
.5
.4
.3
.2
.1
0

Fig. 53 *b*.

Table 55. *Instantaneous growth-rate* (k).

Brody's figures.

Entity in question	Time (days)	Growth-rate % per day (k)	Time in which the entity is doubled (d)	Investigator
Chick				
Wet weight	5–8	56	1·2	Lamson & Edmond
,, ...	8–13	36	1·9	,, ,,
,, ...	13–18	24	2·9	,, ,,
,, ...	6–10	56	1·2	Hasselbalch
,, ...	10–14	29	2·4	,,
,, ...	14–19	19	3·6	,,
,, ...	6–10	47	1·5	Murray
,, ...	10–14	33	2·1	,,
,, ...	14–19	21	3·1	,,
CO_2 excretion ...	0–4	98	0·7	Atwood & Weakley
,, ...	4–14	31	2·2	,, ,,
,, ...	14–19	Pause	—	,, ,,
,, ...	19–21	31	—	,, ,,
,, ...	0–16	36	1·9	Hasselbalch
,, ...	0–16	32	2·2	Murray
Urea excretion ...	5–7	76	—	Needham
,, ...	7–14	34	—	,,
Glutathione content...	6–9	54	—	Murray
,, ...	9–15	30	—	,,
Total CO_2 content ...	7–9	56	—	,,
,, ...	9–16	37	—	,,
Chloride content ...	12–15	32	—	,,
Calcium content ...	12–16	85	—	Plimmer & Lowndes
Creatine content ...	14–21	28	—	Mellanby
Nitrogen content ...	6–9	60	—	Murray
,, ...	10–15	47	—	,,
,, ...	15–20	23	—	,,
Total solid content ...	5–10	57	—	,,
,, ...	10–16	46	—	,,
Ash content	10–14	43	—	,,
,, ...	14–19	25	—	,,
Calorific value ...	7–9	56	—	,,
,, ...	10–15	47	—	,,
Rat				
Wet weight	13–22	53	1·3	Stotsenberg
Guinea-pig				
Wet weight	17–20	100	0·7	Ibsen & Ibsen; Draper; Hensen
,, ...	20–35	25	2·8	,, ,,
,, ...	35–52	9	7·8	,, ,,
,, ...	52–70	5	15·1	,, ,,
Man				
Wet weight	60–110	8	8·7	Streeter
,, ...	110–160	Not straight		,,
,, ...	160–240	1·7	41·0	,,
,, ...	240–280	1·3	55·0	,,

Brody himself did not omit to make suggestions as to possible correlations between his abrupt breaks in growth-rate and other phenomena known to be taking place during the embryonic development of the chick. In the first place, he associated the breaks in the growth-rates of carbon dioxide production at the 14th day with the change in mode of respiration from aquatic to terrestrial which takes place late in incubation. This is quite a convincing correlation, but his suggestion that the first break (at four days), before which the instantaneous growth-rate is about 100 per cent., and the Minot growth-rate 1000 per cent., is associated with a general critical period occurring at that time is not really so satisfactory. For almost any process has its critical moments during development—for example, the peak in protein metabolism at 8·5 days. In cases where there is no *a priori* reason for assuming correlations except the one fact that their peaks coincide or are converse to each other, the utmost caution should be used in so correlating them. Wholesale correlations of apparently unrelated phenomena may be chemically misleading. Thus Brody cites Tomita's peak in total lactic acid content at the 5th day (see Fig. 292) as evidence of a critical period corresponding to the abrupt break in his growth-rates of carbon dioxide production and to the peak in Payne's mortality curve (see Fig. 443).

Brody is not the only investigator who has occupied himself with the growth-rates of different chemical processes and amounts in the embryo, but, before passing on to discuss these points, which will lead naturally to the question of the growth-rates of parts of embryos, a further word must be said about Brody's work.

At present it is not possible to tell much from the comparison of embryos of different kinds, though it is obvious that an immense field of research is opened up here for the comparative embryologist of the future. Thus the equation for the development of the chick embryo in weight according to Murray is $W = 0{\cdot}668t^{3{\cdot}6}$, corresponding to instantaneous growth-constants of 0·47, 0·33, and 0·21 successively,* while the equation for Stotsenberg's rat embryo figures, according to Brody, is $W = 0{\cdot}000065e^{0{\cdot}53t}$, corresponding to a steady rate of 53 per cent. per day instantaneous. On the steadiness of this rate Brody says, "If there is no fallacy in this reasoning we have reached a new and an extremely important conclusion. While all investigators of the time relations of growth have reached the conclusion that the percentage growth-rate continuously and rapidly declines with age, our conclusion is that the instantaneous percentage growth-rate remains constant for the relatively enormously long period between 14 days and birth. The cause of this difference in results is due to the fallacy in the method of analysis employed by Minot". This is only true, subject to confirmation of the fact that the foetal log. weight/age graph gives a straight line over definite periods, and this is just what is not certain. Decision on the matter cannot yet be made.

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* A later value, due to Vladimirov & Danilina, is $W=0{\cdot}324t^{3{\cdot}8}$.

Another interesting point which emerges from Table 55 is the long embryonic stage in the guinea-pig. The chick hatches when its *k* is about 0·21 and the rat is born when its *k* is even higher—0·53, but the guinea-pig stays inside the uterus until its instantaneous percentage growth-rate has dropped to 0·05. Brody succeeded, indeed, in raising guinea-pigs by feeding them on hay and grain immediately after birth, so that they tasted neither colostrum nor milk.

It is interesting, again, to note that there is only one break in the instantaneous growth-rate of carbon dioxide production, whereas there are at least two in the instantaneous growth-rate of wet weight. This must mean either that the respiratory function develops at a rate quite independent of the growth in mass, or that the weight of the body cannot be taken as an index of the growth of the metabolising tissues. This point will be referred to again, for it is of much importance in chemical embryology. On the other hand, the respiration *k* does show a break about the 17th or 18th day, which *is* duplicated in the wet weight *k*, or, at any rate, in the log. weight curve constructed from Lamson & Edmond's data—for it is not so apparent in those of Hasselbalch and of Murray. This may be associated, as we have seen already is Brody's suggestion, with the change in form of respiration occurring then (chorio-allantoic to pulmonary). There is no doubt that some obscure events are associated with this late stage in the chick, e.g. the mortality peak of Payne, which can be greatly intensified if a certain lethal gene is present, and the sudden immunity to implanted rat sarcomata (Murphy), which the chick then acquires. Brody suggests that the chick embryo passes at this stage through a "metamorphosis" similar to those hidden ones which exist, according to Davenport, in the development of man.

The extremely small values of *k* for the embryonic period of man are worth attention. The human embryo grows a great deal more slowly than any other. Five months after conception the instantaneous percentage growth-rate is only 1·7 per day, while, during the week preceding birth, the rat embryo grows at the rate of 53 per cent. per day. The lowest rate of growth ever reached by the rat after birth is 3 per cent. per day. Given percentage rates of growth, therefore, do not signify equivalent stages of development irrespective of the species of animal.

Calculation of the rates of growth for various processes and individual components in the development of the embryo has also been done by other investigators using the Minot method. In 1927 I calculated the percentage growth-rate for the total carbohydrate content of the chick embryo; it fell from 56 to 22 per cent. In Fig. 54 is shown the fall in the Minot curves for the wet weight of the whole embryo, the calorific value, the dry weight of the whole embryo, the sugar, protein, and fat content of the embryo. All of them fall, but we have here an instance of the limited but real value of the Minot curves, which, although no absolute conclusion can be drawn from them, do show that the tissue constituents and the dry weight have a different behaviour from the wet weight. It can easily be seen that they form a plateau between the 10th and the 15th day, during which they grow at a constant rate while the wet weight is falling all the time. This plateau also appears on the curves for carbon dioxide output calculated in the same manner as percentage growth-rates from the figures of Bohr & Hasselbalch; Atwood & Weakley; and Murray in 1927 by Brody. The plateau must be due to the fact that the growth of dry substance is specially rapid during the middle phase of development; it is then that the embryo makes the most rapid strides from wetness to dryness. It is interesting to see that the growth-rate of carbohydrate is never as high as some of the others, and never drops so low. It is significant, moreover, that on the 19th day the Minot growth-rate of the protein has dropped below that of the whole body, while the growth-rate of fat remains well above it. This is an illustration of the "relative" use of Minot's method.

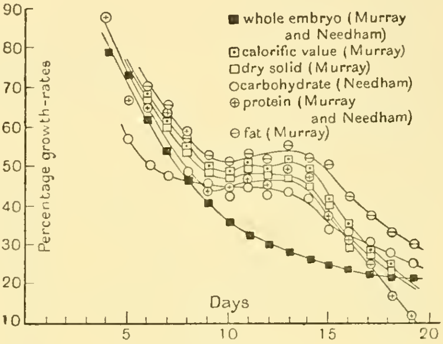


Fig. 54.

2·9. Growth Constants

Brody is not the only worker who has applied the differential calculus to embryonic growth-curves. Teissier and Lambert & Teissier suggested simultaneously that this should be done, but their work was quite theoretical. However, Schmalhausen published independently at almost exactly the same time a paper in which it actually was done. He criticised Minot's method of calculating "mittlerer prozentualer Zuwachs" in arbitrary time-units from exactly the same point of view as Brody. Thus, he says of the Minot method: "the larger time intervals we take, the bigger the error will be. With equal time intervals, the error will be bigger the bigger the rapidity of growth, and this will in fact lead to altogether misleading figures for the early periods". We need not follow Schmalhausen's reasoning, which led him to adopt the calculus as a better assistance in studying growth-curves, for we have already examined and approved the

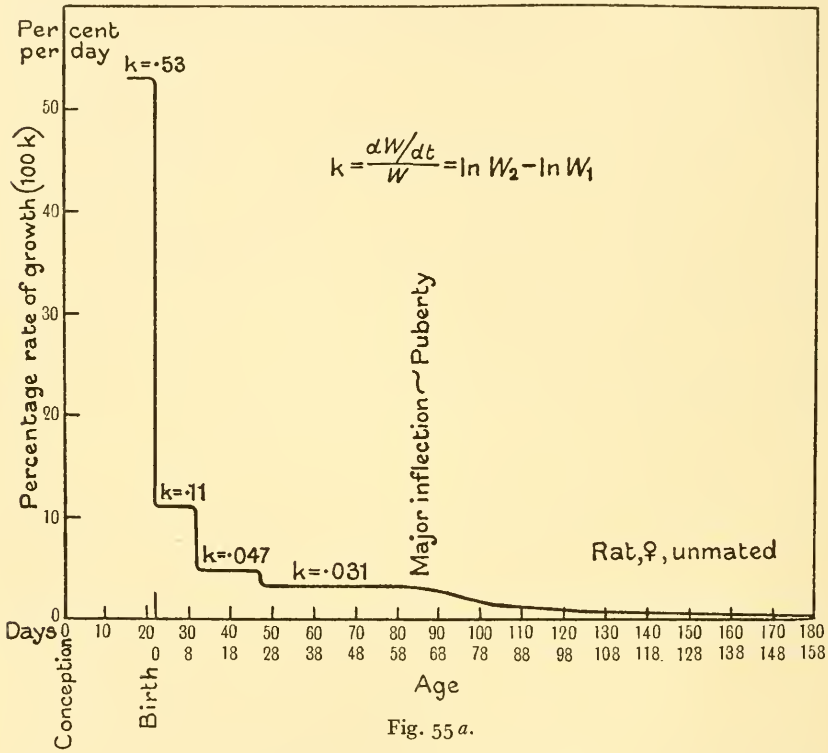
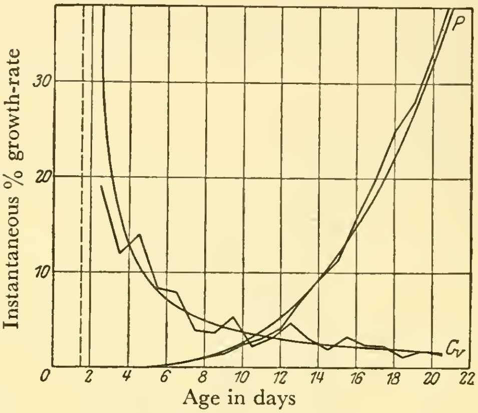


Fig. 55 *a*.

arguments of Brody. Schmalhausen speaks of the "wahre Wachstumsgeschwindigkeit" instead of the instantaneous percentage growth-rate, and of *r* instead of *k*. His equation relating the instantaneous percentage growth-rate to the Minot growth-rate is exactly the same as Brody's, and he points out that, when, according to the old method, the growth-rate would be 700, the instantaneous method would give a result of 207, though 50 per cent. (Minot) would be equivalent to 40·5 (instantaneous). These figures might have been read off from the graph of relation given by Brody, and it is surprising that the two workers, one at Kiev and the other in Missouri, should have been thinking on such very similar lines. It is still more surprising that embryologists had not thought on such lines long before. Schmalhausen and Brody diverge, however, upon one important point, namely, the shape of the line given when the log. weight is plotted against the age, for Schmalhausen regards it as a curve—just as Murray and McDowell do—while, as we have seen, Brody lays great stress on the representation of it by a series of straight lines having abrupt breaks between them. Thus, the instantaneous percentage growth-rate, which with Brody remains constant over certain definite periods, with Schmalhausen continually declines in value. In other words, Brody's diagram which shows the instantaneous percentage growth-rate dropping in a stepped formation from fertilisation to hatching is replaced in Schmalhausen's work by a regular curve passing downwards to become asymptotic to the abscissa (Fig. 55 *b*), just as the Minot curve does, only, of course, plotted from a set of figures having a real meaning as against the abstractions of Minot. This difference of outlook leads naturally to very wide differences in conclusions; thus Schmalhausen has nothing to say about critical points or hidden metamorphoses. Having diverged from Brody in this direction,



P = wet weight;
Cv = instantaneous % growth-rate.

Fig. 55 *b*.

he proceeded a good deal further along it by observing that the graph relating instantaneous percentage growth-rate to age was practically identical with a rectangular hyperbola, and that there was a simple relation between the values of r or Cv (Brody's k) and the age, for the product of the two was always roughly equal to 300.*

Table 56. *Embryonic growth: Schmalhausen's "Wachstumskonstante" ("wahre Wachstumsgeschwindigkeit" × time).*

		Cvl length	Cvt age	Investigator
MAN	Whole embryo wet weight	193	369	Friedenthal
MOUSE	,, ,,	—	337·5	McDowell *et al.*
RAT	,, ,,	441	518	Stotsenberg
CHICK	,, ,,	—	318·5	Murray
,,	,, ,,	518	321	Schmalhausen
,,	Liver	—	329	,,
,,	Lung	—	321	,,
,,	Fore limb (whole period)	—	293	,,
,,	,, (3–18 days) ...	—	329	,,
,,	Hind limb (whole period)	—	347	,,
,,	,, (3–18 days) ...	—	395	,,
,,	Stomach	—	374	,,
,,	Brain	—	210	,,
,,	Lens	—	210	,,
,,	Whole eye (2–10 days) ...	—	317	,,
,,	,, (11–21 days) ...	—	99	,,
,,	Heart	—	276	,,
,,	Mesonephros (4–13 days)	—	224·5	,,
,,	Metanephros (7–17 days)	—	359	,,
,,	,, (17–21 days)	—	196	,,
,,	Ovary	—	145	,,
GUINEA-PIG	Whole embryo wet weight	—	347	Draper
TROUT	,, ,,			
	(30–51 days)	—	206	Kronfeld & Scheminzki
	(51–99 days)	—	207	,, ,,

Schmalhausen gives no explanation of the breaks in the cases of those organs which have two values of Cvt, but calls attention to the fact that the organs of early differentiation have low Cvt and *vice versâ*.

If the curve obtained by plotting Cv (Brody's k) against time is a regular hyperbola, then the product Cvt should be 300. If it exceeds this figure, the curve is descending and becoming asymptotic less rapidly, i.e. the rate of growth (instantaneous) is not falling off as rapidly as it will be if the product is less than 300 at any given moment.

This constant he calls the "Wachstumskonstante", and its values, calculated by him for a number of embryonic processes, are seen in Table 56. It is perhaps the least convincing part of his exposition, for when during a certain series, e.g. the growth of the human embryo, the constant Cvt oscillates between 899 and 93 as extreme limits, one may legitimately doubt whether great stress can be laid on the average. Moreover, Janisch treats the same curve as a catenary exponential one. And, although the "Wachstumskonstante" for the various organs and parts of the embryo show differences which might well be regarded as characteristic for the tissue in question, it is disturbing to find so wide a difference from the predicted value in the case of the rat embryo, explained though it is by Schmalhausen as due to variable factors in the food of the maternal organism. The reason why 300 is the number to which these figures approach is, of course, because, according to Schmalhausen's formula, the increase of the embryonic weight can be expressed by the equation

---

* Brody himself does not find this to be so.

$$W = k\,(at)^3,$$

where W is the weight, a the "Lineargrösse", t the time and k a constant. This agrees with the hyperbolic nature of the Cv/t curve,

Table 57. *Instantaneous percentage growth-rate* (*Chick*).

Day of development	BRODY (Smoothed)	SCHMALHAUSEN	
		(Raw)	(Smoothed)
0–1	—	—	—
1–2	—	—	—
2–3	—	190	190
3–4	—	119	140
4–5	—	139	107
5–6	—	83	87
6–7	47	79	70
7–8	47	38	60
8–9	47	36	50
9–10	33	53	42
10–11	33	22	38
11–12	33	31	33
12–13	33	48	30
13–14	33	30	27
14–15	21	20	25
15–16	21	32	23
16–17	21	23	21
17–18	21	22	19
18–19	21	11	17
19–20	—	17	15
20–21	—	16	12

for the equation of an equal-sided hyperbola is $y = 3/x$. Schmalhausen does not derive his Cv directly, but calculates it in each case from the Minot percentage growth-rate figures. It is instructive to place side by side the instantaneous percentage growth-rates of Brody and Schmalhausen for the chick embryo, as is done in Table 57. That of the former has three constant periods, that of the latter shows a gradual decline, and the figures illustrate what has already been said, namely, that, until we possess much better statistical data than is actually the case, we cannot differentiate between the Brody position and the Murray-McDowell-Schmalhausen position.* As the matter is fundamental in view of the important theoretical issues involved, the accumulation of more data is urgently to be desired. It may be mentioned that Cohn & Murray, plotting log. weight/age curves for the growth of embryonic heart cells in tissue culture, obtained curves concave to the age axis and not straight lines.

Schmalhausen also studied the growth in length of the chick embryo, calculating it from the weight by the formula $L = \sqrt[3]{W}$. The daily gain in length *a* he found to be variable around a constant value of 1·47 for the first half of development and 2·00 for the second half. But when the weights were corrected by the estimation of the embryo's specific gravity (average for first half 1·025, average for second half 1·06) the corresponding daily gains in length worked out at 1·60 and 1·79. A further correction made necessary by the presence of the feathers during the last half of development brought the figure down to 1·64, so that throughout incubation the embryo apparently grows in length at the same average rate. The duck embryo, according to Schmalhausen, has a daily length increment of 1·10 mm., and this value he regards as constant for the species. He went on to calculate *a* for the human embryo, using the weight data of Friedenthal and Zangemeister, and for the embryo of the white rat, using the data of Stotsenberg. All these results are shown in Table 58, together with his further assessments of *a* calculated from the "Normaltafeln" of Minot & Taylor for the rabbit and Keibel for the pig. The daily size ("Lineargrösse") increments of his own measurements of separate organs and parts of the chick embryo are also given. During the course of development the value of *a* rises and falls according to the rate of growth. If the value a/l is calculated, where *a* is the daily increment in length and *l* the length of the part in question at the beginning of the period, the absolute size of the part will cease to affect the result, and the organs will be comparable with themselves and with the whole embryo. When this is done, the ratio is found to be fairly constant, rising as high as 10·3 per cent. for the stomach and falling as low as 6·3 per cent. for the lens. These figures are also given in Table 58. Schmalhausen concluded from them that organs which reach a high state of differentiation early grow the most slowly (brain and lens), while less differentiated organs grow most quickly (liver and limb-buds). The growth of the body as a whole is the average practically exactly of the rest, and it is interesting to note that the organ which most nearly approaches it is the heart. The heart would seem to grow in size at the same rate as the entire body. But, as Schmalhausen says, this growth in size seems to have no simple relation to the growth in weight as shown by the percentage growth-rate.

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* Recent work by Byerly supports that of Brody.

Table 58. *Schmalhausen's values for* a, *i.e. daily increment in size or "Lineargrösse".*

		Millimetres		
		a	*a/l*	Investigator
CHICK	Whole embryo (1st half)	1·6	—	Schmalhausen
,,	,, (2nd half)	1·64	7·65	,,
,,	Brain	0·5	6·50	,,
,,	Lens	0·1	6·30	,,
,,	Spleen	0·1	6·62	,,
,,	Heart	0·3	7·50	,,
,,	Lung	0·29	7·82	,,
,,	Liver	0·55	8·83	,,
,,	Testis	0·09	8·82	,,
,,	Metanephros	0·32	10·10	,,
,,	Stomach	0·64	10·30	,,
,,	Fore limb	0·44	7·33	,,
,,	Hind limb	0·76	8·85	,,
,,	Pectoral muscles	0·43	9·09	,,
DUCK	Whole embryo	1·10	—	,,
MAN	,,	0·55	—	Friedenthal
,,	,,	0·55	—	Zangemeister
RAT	,,	1·47	—	Stotsenberg
RABBIT	,,	1·2	—	Minot & Taylor
PIG	,,	1·18	—	Keibel
GUINEA-PIG	,,	0·75	—	Read

In a later paper Schmalhausen studied the relation between initial weight and end weight in a number of animals, wishing to obtain some means of comparing their "Wachstumserträge" or Growth-yields, on a basis independent of their size. He found that u, or the mass of substance added on to itself by the organism between times t_1 and t_2, could be calculated by the formula

$$u = \int_{t_1}^{t_2} \frac{k}{t}\,dt = kln\frac{t_2}{t_1} = k\log\frac{t_2}{t_1} \times \frac{1}{0{\cdot}4343},$$

the initial weight being taken as unity ($k = Cvt$). His results for various organisms show interesting differences, thus:

	u embryonic period	u post-embryonic period	u whole life-span
Sturgeon	2·2	17·2	19·4
Pike	1·8	15·3	17·1
Hen	9·5	3·8	13·3
Mouse	8·9	2·3	11·2
Rat	13·6	4·3	17·9
Guinea-pig	13·6	2·3	15·9
Pig	15·6	4·8	20·5
Man	20·9	3·3	24·3

Here we observe the effect of early hatching in the two aquatic forms, which have the greater part of their growth still before them at the time of leaving the egg. The other figures demonstrate quantitatively what is apparent to common sense, namely, that the embryonic period is the time of greatest growth in terrestrial animals.

2·10. The Growth of Parts

We must now turn to the relative growth-rates of parts of the embryonic organism. This is a field which has mainly been tilled by anatomists, but it is of the greatest importance to the chemical embryologist. For the increasing and decreasing intensities of physicochemical processes cannot be intelligently studied in the absence of a knowledge of the distribution of the whole mass among the different organs and tissues. The investigation of the relative growths of endocrine glands, again, cannot but throw much light on the development of the adult metabolism in the embryo. D'Arcy Thompson sees an appreciation of this in the eighteenth-century preformationists. "It was the apparently unlimited extent", he says, "to which, in the development of the chick embryo, inequalities of growth could and did produce changes of form and changes of anatomical structure that led Haller to surmise that the process was actually without limits and that all development was but an unfolding, an '*evolutio*' in which no part came into being which had not essentially existed before. In short the celebrated doctrine of preformation implied on the one hand a clear recognition of what, throughout the later stages of development, growth can do, by hastening the increase in size in one part, hindering that of another, changing their relative magnitudes and positions, and altering their forms; while on the other hand, it betrayed a failure—

inevitable in those days—to recognise the essential difference between these movements of masses and the molecular processes which precede and accompany them and which are characteristic of another order of magnitude."

The papers of Schmalhausen are of much importance in this matter. Inspired by the views of His, who declared in 1874 that all the development of shape could be ascribed to unequal growth in various component parts of the embryo, he set himself to weigh and measure a great number of these individual sections.

He first studied the relative growth-rates of the brain and eye of the chick embryo, together with the liver, lung and stomach, representing the organs of endodermal origin. In each case, he calculated the % growth-rates and the percentages formed of the weight of the whole body. For the organs of mesodermal origin, he chose the heart, the mesonephros, the metanephros, the ovary, and testis. These figures he treated in the same way. In many cases his weights were not obtained directly but by reconstructing from serial sections and then weighing, proper allowance being made for complicating factors such as specific gravity. Fig. 56 shows one of his graphs—it is specially interesting as showing the definite decrease in weight which the mesonephros undergoes after the 15th day in giving place to the metanephros or adult kidney. It also includes % growth-rate curves for the fore and hind limbs. Lastly, he ascertained the growth-rate of the feathers.

In general, he found that the changes in the growth-rates of organs were synchronous. The percentage growth-rate (see Fig. 57) seemed to have peaks in its descent, each one less marked than the preceding one. In each case, the growth-rate of every organ shows a certain rise, but the amount of the rise differs in different cases—thus the lung is the organ which is growing fastest about the 6th day, the hind extremity about the 10th day and the stomach about the 13th. On the whole, the periods of depression of the growth-rate of the majority of organs are from 7 to 9 days, from 10½ to 11½ days, and from 14 to 16 days. When the weights of individual organs, however, were arranged plotted against weight of embryo, not age, the peaks disappeared, as would be expected, for the total weight is the sum of the weights of the organs. It would be interesting to plot the logs. of Schmalhausen's organ-weights against age in order to obtain the instantaneous growth-constants of Brody for each one. Schmalhausen's general results, however, were as follows: on the 5th and 6th days, the growth-rate of all organs is falling, with the possible exception of liver and hind limb. This continues till the beginning of the 7th day, save that the eye and the lens may show a slight rise. At the beginning of the 7th day, however, the growth-rates of all organs rise, firstly the mesonephros, the liver and the lung, and, to a less extent, the hind limbs, then these are followed by the rest. After a peak all fall until the 11th day, when all again rise, only to fall on the 14th, with the exception of the feathers, which maintain a rise. Later a more gradual rise in growth-rate takes place throughout the body, begun by the lens and liver and, to a less degree, by the eye and the brain, and continued by the stomach and the mesonephros. After the subsequent fall, only small variations take place, which are found to be synchronous for groups of organs such as kidney-liver-

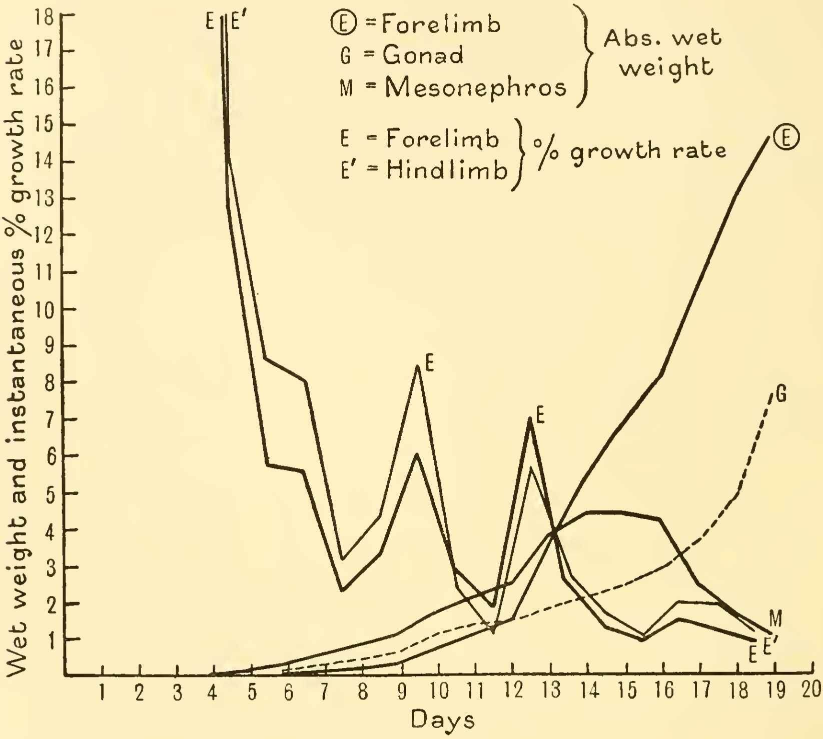

Fig. 56.

stomach until the end of the embryonic period. It is certainly interesting that organs so different in origin and nature as the eye and the mesonephros should be similarly affected by spurts of growth at various stages, and Schmalhausen concluded that this was an argument against the hypothesis of specific organ-stimulating substances, the presence of which would from time to time cause more rapid growth in one place of the embryo than in another. It looks much more as if growth-promoting substances were passed round in the embryonic circulation at certain definite intervals, and so exercised an effect on a large number of different organs. In this connection, the recent work on the growth-promoting substances of egg-yolk should be borne in mind, and the experimentally determined cycles of varying permeability to fat-soluble and water-soluble substances in the walls of the vitelline blood-vessels. One relation which seems clear from Schmalhausen's work is that growth

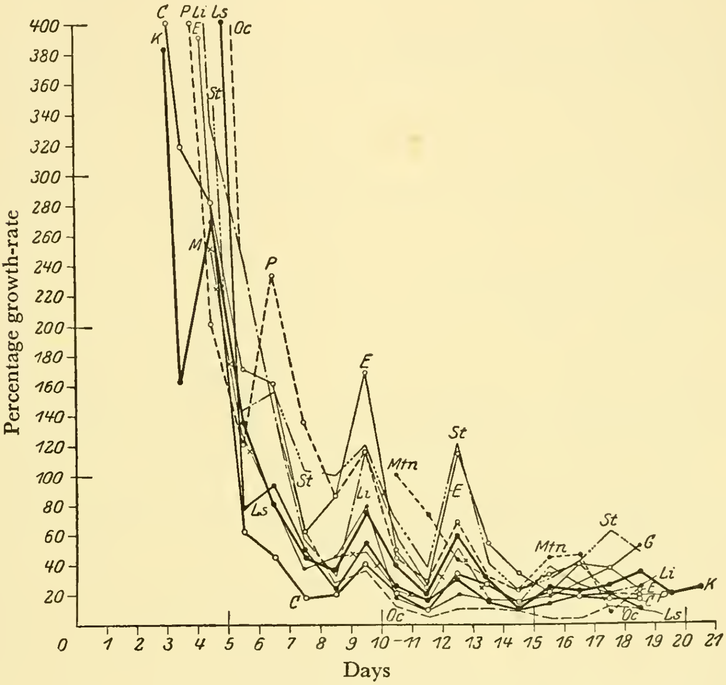

Fig. 57. C=brain; E=hind limb; K=whole body; Li=liver; Ls=lens; M=mesonephros; Oc=eye; P=lung; Mtn=metanephros; St=stomach; G=gonads.

of fore and hind limbs does not accomplish itself in the same spurts as the viscera do, for during the 8th day depression in the growth-rates of the latter the skeleton is growing vigorously, and during the 10th day peak it rather falls off. Very similar remarks apply to the hind limb growth-rate. Schmalhausen concluded that very young organs can respond to a given intra-embryonic environment by increase in growth-intensity, while more differentiated organs can at the same time respond by depressions in their growth-rates. "If one and the same influence", he says, "can act in a stimulatory manner on the growth of some parts or organs, and inhibitorily on the growth of others, we can see how unequal growth can take place and hence a change in form." It would also appear that the more development goes on, the more different the rates of growth of different organs are. Three factors seem to control the growth-rate of a single organ: (1) the age of the embryo, (2) its own degree of differentiation, and (3) growth-promoting substances or embryonic hormones present in the circulation. Under (2) would be included the time of origin of its "anlage" and the intensity with which its preliminary growth would take place. These views are not compatible with Mehnert's "laws of organogenesis", the main one of which was that the growth-rate of an organ in the embryo was proportional to its degree of development at the time in question. The only criticism that can be levelled against Schmalhausen's work is that the number of embryos employed was perhaps rather few.

In conjunction with Stepanova, Schmalhausen made further investigations on the growth of the embryonic skeleton in the chick. Similar fluctuations in pre-natal growth-rates of parts have been discussed as regards the primates by Schultz.

Schmalhausen has attempted to give an explanation of these spurts in terms of metabolism. Summarised again there are, in the case of the chick, three or four periods, in each of which the growth-rate first rises and then falls, as follows:

Days	
0–4	1st period, great fall from a high value
4–9	2nd period, rising to the 6th day then falling
9–12	3rd period, rising to the 10th day then falling
12–15	4th period, rising to the 13th day then falling
15–21	5th period, rising to the 17th day then falling

He has suggested that these periods may partly correspond to the periods which can be distinguished in the development of the chick embryo, during which one type of chemical molecule is predominantly burned to furnish energy for the growing organism. This subject will be handled fully later (Sections 6·8, 7·7, and 9·5); here it suffices to say that the beginning of development is in many ways closely associated with an important carbohydrate metabolism, and the latter part with the metabolism of fatty acids, while the intermediate part would appear to have an association with catabolism of protein, in view of the fact that the point of maximum protein catabolism occurs when 8·5 days of development have been completed. These periods, in Schmalhausen's view, may be identified with those in which he finds spurts in the growth-rate. A certain amount of scepticism about this identification would seem justifiable until we have irrefragable proof that the spurts are more than chance variations in a curve composed of too few data. His suggestions involve the view that "Abbauprodukte" accumulate from time to time in the developing embryo, and so hinder its growth (essentially the same theory as those of Jickeli and of Montgomery). Thus his first depression of the growth on the 4th day corresponds to an accumulation of lactic acid and ammonia (see further on for the detailed references) and his second depression of growth on the 9th day corresponds to an accumulation of urea. Finally, his third depression of growth about the 12th day corresponds to an accumulation of uric acid. He admits that there is nothing chemical which obviously coincides with the later depressions of growth, but supposes that they depend on the decreasing excretory power of the mesonephros. After the 16th day the metanephros would be undertaking the duty of excreting waste products, and growth accordingly begins again. Ingenious as these correlations are, they cannot be said to be convincing, in view of the fact that many other processes besides the excretion of waste products may be supposed to be exercising an effect on the growth-rate. More interesting is Schmalhausen's attribution of great importance to the surface of the blastoderm, the blastodermal capillaries, and the active surface of the excretory organs. Measurement of these during the course of development would throw a bright light on these problems. Schmalhausen did himself compare the growth in weight of the embryonic kidneys with the daily increment of the whole body, and, although the figures were rather erratic, he felt able to conclude that, owing to the slow growth of the mesonephros and metanephros, the excretory surface was only just keeping pace with the growth of the embryo. In these circumstances, it was not surprising to find now an accumulation and now a flushing out of waste products from the embryonic body. In a later paper, however, he modified considerably his views on this subject.

As regards the growth of individual parts, Schmalhausen later introduced several further expressions. "Homonomic growth", in his terminology, means growth of an organism in which all the parts and organs have the same growth-constant, "heteronomic growth" —the more usual form—is the growth of an organism composed of organs each with its own characteristic growth-constant. Then the growth-directing force may exist either inside or outside the anlages of the separate organs—in the chick it apparently does not exist inside—and in the former case it would be called "autonomic growth", in the latter "automorphic", while, if the influence was directly the growth of another organ, it would be termed "heteromorphic". Schmalhausen found that, although the organs in the chick embryo taken at any one moment had very different rates of growth, yet, if they were all dated, as it were, from the time of formation of their anlages, they showed very similar rates of growth. Thus an anlage developing late would be growing much quicker than the whole body, while, at the same time, if its instantaneous percentage growth-rate curve was plotted, it would be found to have a shape very like that of the organism as a whole. Thus organs can only be compared as to their growth-rates if they are taken from their own particular origins and not from the origin of the body as a whole. In homonomic growth, of course, one is dealing with organs originating at the same time and having identical growth-constants. In this case, the definite proportions of the resulting organism can be deduced from those of the anlages; in other words, a kind of preformation holds good.

If Table 56 be again referred to, it will be seen that, on the whole, it takes functioning organs longer to grow than functionless ones. Thus the metanephros, which at first has a *Cvt* of 359, drops to 196 after it has begun to excrete actively by about the 16th day. In Fig. 58 is shown the relation between the weights of the organs in the embryo chick expressed as percentages of the total weight of the body. The heart and mesonephros are seen to have their maximal relative size very early in development, after which the former declines slowly and the latter more rapidly. The first four days of development see also the maximal relative size of brain and lens, but these fall very rapidly away from their pre-eminence. Towards the end of the developmental period, the fore limb gains much in importance, and about that time also the metanephros reaches a maximal point of growth.

For further comparison further calculations are necessary. The relative instantaneous percentage growth-rate could be obtained from the equation

$$\frac{Cv}{Cw} = \frac{\log v_1 - \log v}{\log w_1 - \log w},$$

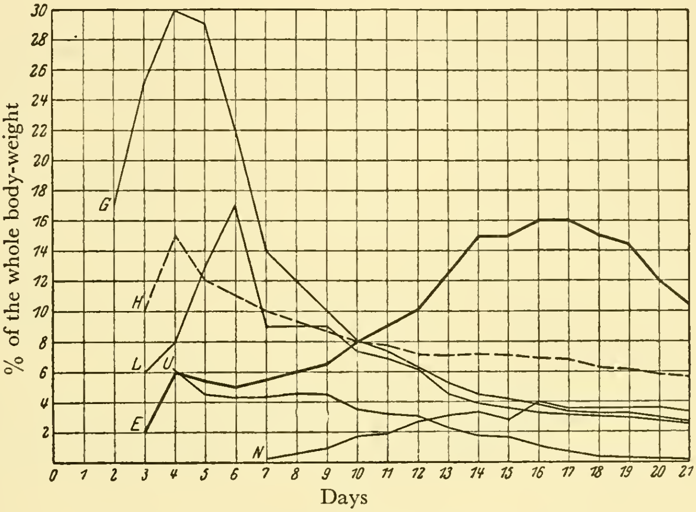

Fig. 58. E = fore limb; G = brain; H = heart; L = lens; N = metanephros; U = mesonephros.

where Cv is the instantaneous percentage growth-rate for the organ or part in question, v_1 and v the weights of the organ at the beginning and end of the period in question, Cw the instantaneous percentage growth-rate of the organism as a whole and w_1 and w the weights of the organism as a whole at the beginning and end of the period in question. But this would not take into account the time of formation of the various anlages. More complicated expressions have, therefore, to be found, but as they do not at present seem to have any direct importance for the chemical embryologist, a reference to the original paper must suffice. They involve the computation of an "extensity factor" which is usually the same as the constant *a*, already referred to, and an intensity factor which is the corrected product of the instantaneous percentage growth-rate and the time, i.e. *Cvt*. The relative extensity factor is obtained by determining the time which is taken by the organism or the organ to grow 1 mm. in length, thus reversing the process by which *a* was originally found. The size of the anlage is also included, and called the mass factor. By the aid of all this apparatus, Schmalhausen compares organs on a common basis, i.e. the time taken for 1 mm. increase in length to be made. Thus the extensity factor of the chick embryo brain (*r*) is 1·27 and that of the duck embryo brain 1·26, which means that the sizes of the respective organs are in their earliest stages almost identical. But the relative instantaneous percentage growth-rate (intensity factor, *k*, or *Cv*) differs considerably, for in the chick it is 1·87 and in the duck 2·01, which means that the duck embryo brain grows distinctly more rapidly than that of the chick embryo and finally attains a larger size. Again, for the stomach of the chick embryo the extensity factor, *r*, is 0·244 and for the duck 0·324, but the intensity factor is 3·59 for the chick and 2·86 for the duck, or, in unquantitative terms, the stomach is rather bigger to start with (relatively) in the duck than in the hen, but the chick stomach grows faster and reaches eventually a larger proportion of the body.

This work on disproportionate or heterogonic growth led Schmalhausen into a field which had been in course of investigation by Huxley and others. Schmalhausen was able to obtain Huxley's formula from his own, and concluded with some justice that his own were of fairly general validity and did not hold only for embryonic growth. On the other hand, owing to the absence of a true extensity factor in Huxley's formula, the latter could not be applied to the embryo; for, although in post-embryonic growth-curves the ages of all the organs can be taken as approximately identical with the age of the animal, this is by no means the case in embryological work, where the time of formation of the various "anlages" is of the greatest importance. The investigations on heterogonic growth are not immediately germane to the theme of this book, but they may at any moment become very important for the chemical physiology of the embryo, and it is necessary therefore to be aware of them. Schmalhausen's work is really an extension to the embryo of the conceptions of Pézard and Champy, as worked out in recent years by Huxley and his associates. A rich harvest awaits the investigator who discovers the relation between chemical constitution and the differential growth-ratios. Perhaps a fruitful line of work will develop from the finding of Robb that the log. weight of an organ plotted against the log. body-weight often gives a straight line. He has suggested that organ-growth may depend on a kind of partition-coefficient, organs competing, as it were, for the building-stones in the blood-stream, and securing now a greater now a lesser proportion, according to the changing permeability of their cell-walls.

The changes which occur in the chick's relative growth-rates of parts at hatching have been studied by Latimer, who combined together the data collected for pre-natal stages by Schmalhausen and those for post-natal stages by various American workers. His results lead to the conclusion that the organs and parts fall into three groups:

(i) Those in which no change in relative growth-rate is found, e.g. liver, gizzard, feathers, ovaries.

(ii) Those which show a brief post-natal retardation, e.g. total body-weight, brain and heart.

(iii) Those which show a marked post-natal acceleration, e.g. kidneys and spleen.

As will be shown below, the brain and eyes, so prominent in the embryo, fall consistently throughout life when expressed as per cent. of the whole weight, while the gizzard, liver, kidneys, spleen and heart have a maximum in early post-natal life.

Other work on relative sizes of parts has been done by Jenkinson on embryonic trout, by Keene & Hewer on man, and by Jackson who gives a graph (Fig. 59) showing the relative proportions in the human embryo at different stages of its development, collected from all the available data. Boyd (on man) and Welcker & Brandt (on the chick, salamander and man) made earlier attempts at the same thing, but the ages of their embryos were unknown. The graph demonstrates the relatively large size of the brain in the early sizes, and in many ways resembles the graph for the organs of the chick given by Schmalhausen. "In general," says Jackson, "the period of maximum relative growth passes in a somewhat wave-like manner over the body from the head towards the foot. The head reaches its maximum relative size about the 2nd month. In the trunk, the upper portion, including the thorax and the upper abdominal viscera, is relatively largest throughout the earlier half of foetal life.

The lower part of the abdomen becomes more prominent towards the end of the foetal period, due chiefly to the rapid expansion of the intestines at this time. The pelvis and lower extremities do not reach their greatest relative size until early adult life, although the upper extremities have reached their maximum relative size at birth. It may also be noted that the organs lying dorsal to the body axis grow at first far more rapidly than those ventral to the body axis, for, while in the 2nd month the former are three times the size of the latter, at birth they are equal, and in the adult the latter are six times the size of the former." Jackson's data on the growth of such organs as the suprarenal gland should also be of much service to chemical embryologists, and his paper as a whole is of great value, as it summarises the results of all the earlier workers—Welcker & Brandt; Brandt; Anderson; Lomer; Meeh; Liman; Thoma; Oppenheimer; Collin, Lucien & Beneke; Devergie; Schmidtt and Elsässer. The general results of all the workers who have occupied themselves with the weights of foetal parts are tabulated in Table 59, which gives the point in development at which the maximum percentage of the total body-weight is reached, what that percentage is, what it becomes at birth and what eventually it is in the adult animal.

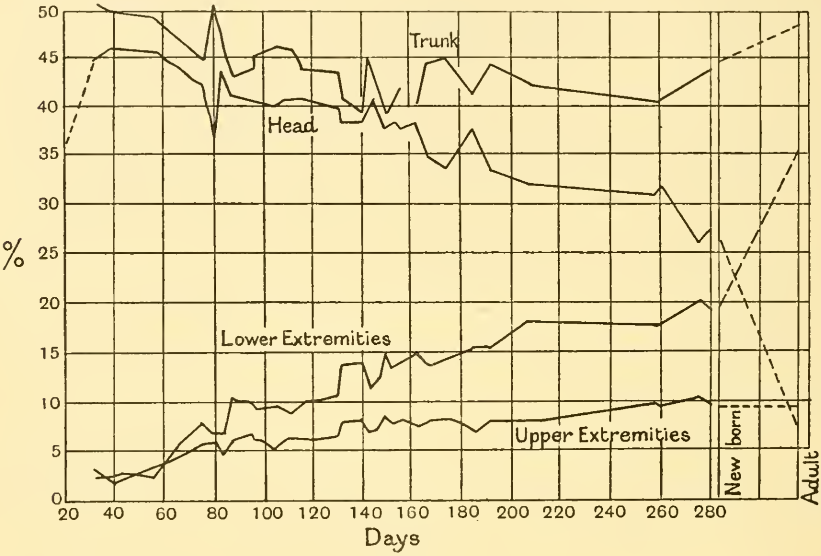

Fig. 59.

Table 59. *Relative maximal weights of constituent parts of the embryo.*

	Part or organ	Point at which maximum is attained in development	% of total body-weight		
			At maximum	At birth or hatching	At adult stage
Pig	Total viscera	15 (length in	38	16·0	8
(Lowrey)	Head	18 mm.)	30	22·0	6
	Brain	18	9	4·0	0·087
	Spinal cord	18	1·87	0·33	0·04
	Eyeball	86	1·15	0·4	0·01
	Heart	15	4·64	1·0	0·32
	Lungs	86	3·9	2·0	0·7
	Liver	25	15·9	3·1	1·38
	Kidneys	58	2·59	1·01	0·25
	Mesonephros	15	12·0	—	—
	Spleen	15	—	0·17	0·13
	Pancreas	15	—	0·16	0·14
	Thymus	15	—	0·37	—
	Thyroid	58	—	0·026	0·004
	Suprarenal	58	0·13	0·019	0·005
	Intest. and stomach	15	—	3·6	4·79
Man	Head	2 (months)	45	26·0	—
(Jackson*)	Trunk	1	65	40·0	—
	Fore limb	10	10	10·0	72·4
	Hind limb	10	20	20·0	
	Brain	2	20	13·5	—
	Spinal cord	1	5	0·15	—
	Heart	1	5	0·7	—
	Liver	2·5	7·5	5·0	—
	Lungs	4	3·3	2·0	0·94
	Spleen	10	0·4	0·4	
	Thymus	10	0·3	0·3	—
	Thyroid	10	0·12	0·12	—
	Kidneys	7	1·0	1·05	0·90
	Suprarenal	3	0·45	0·24	
Chick	Heart	4 (days)	1·5	0·56	—
(Schmalhausen)	Mesonephros	4	0·61	0·014	—
	Metanephros	16	0·39	0·23	—
	Brain	4	30·0	2·6	—
	Lens	6	0·17	0·025	—
	Fore limb	16	3·2	2·1	—
	Stomach	20	3·59	3·56	—
Dogfish	Head	0·09 (wt. in	40·0	17·5	12
(Kearney†)	Skin (birth)	75·0 gm.)	11·3	11·3	7
	Skeleton	85	10·0	8·6	9
	Muscles (adult)	—	63·0	45·0	63
	Total viscera, (a) *c.*	0·1	19·0	12·0	—
	two maxima (b) *c.*	350·0	14·3	—	9·8
	Brain	0·1	15·0	1·6	0·9
	Spinal cord	0·1	1·76	0·5	0·17
	Eyeballs	0·1	9·0	2·0	0·64
	Heart	0·1	4·0	0·15	0·20
	Pancreas	350·0	0·14	0·06	0·14
	Liver	20·0	7·0	4·8	5·9
	Spleen	350·0	0·38	0·098	0·2
	Rectal gland	0·1	0·105	0·032	—
	Mesonephros	1·8	4·8	1·1	0·38
	Testes and ovaries	200·0	0·9	0·4	0·28
	Stomach and intest.	350·0	5·5	2·9	4·0

* Including all previous work on man.

† "The various organs and parts in dogfish [*Mustelus canis*] embryos and adults show relative growths strikingly similar to that which has been observed among the higher vertebrates, including mammals and man".

From this it can be seen that the organs which reach their maximum relative weight early in development are the heart, spleen, pancreas, thymus, brain, spinal cord and head. The mesonephros also, of course, reaches its maximum fairly soon and declines more or less rapidly afterwards. The muscle masses, shown especially in the figures for fore and hind limbs, increase steadily in relative weight and reach their maximal relative size at or shortly before birth. The suprarenal gland, the stomach, the lungs, and the thyroid are variable in their point of maximum. But, as can be seen from the table, the data on these matters are not very numerous, most of the attention which has been given to the relative weights of parts and organs having gone into the study of post-natal life. W. Schultze has made an interesting investigation on the effect of hormones on the developing parts and organs in the tadpole.

It is interesting that the only conclusions to which Jackson would commit himself were (1) that the embryo grows much faster in the earlier stages than in the later, and (2) that, at any rate as far as vertebrates were concerned, pre-natal growth is relatively much greater at the cephalic than at the caudal end. These points had already both been stated by Aristotle, and the whole advance lay in giving them a quantitative backing. Jackson did not consider that his figures supported the view of Preyer that those organs grow fastest in the embryo which will afterwards first come into functional operation.

A great mass of such data has since Jackson's time been collected by Calkins & Scammon and many investigators working under their influence. We need not do more than mention their work on the growth of the spinal axis in the human embryo, that of Scammon on the height-weight index, Nañagas on anencephalic embryos, and Brody and Hammond on proportions in the cow, for these and many others only indirectly concern us. But it is important to note that for the human embryo Calkins & Scammon found that from 3 months onwards the growth in length, girth and diameter of the various external divisions of the body was directly proportional to the growth in total body-length. While each dimension has its own growth-rate with respect to the total body-length, this characteristic rate does not alter during the period under consideration. All these entities then may be expressed by the Calkins equation

$$D = aL \pm b,$$

where *D* is the dimension in question, *L* the total body-length, and *a* and *b* constants. The constant *b* is a measure of the amount of growth that has gone on prior to the period in question, and, as it is negative for the limbs but positive for all head and neck measurements, the conclusion is that while the latter have been growing extremely rapidly before the period began the former have not. This is as would be expected. The same conclusion emerges from the data of Corrado on the weights of head, trunk and extremities as analysed by Scammon.

Many organs have been examined by the investigators of this school. The cerebellum, for instance, was found by Scammon & Dunn to increase in absolute volume and weight first slowly and then more rapidly during the foetal period; thus, its percentage growth-rate rose for the first six months of pregnancy, only to fall sharply afterwards. The pancreas, studied by Scammon, grows at a rate very like that of the whole embryo, but the relative weight of the organ with respect to total body-weight undergoes a reduction from 0·3 per cent. at the 4th month to 0·1 per cent. at birth. The uterus, on the other hand, passes through two definite phases in pre-natal life. Until 7 months the organ shows a lineal increase with respect to body-length which is comparable to that of most lineal body-dimensions, but after 7 months it grows much more rapidly. In early post-natal life, however, the organ goes through an involution stage which has long been known, actually decreasing in size by hypoplasia and hypotrophy until it reaches the level it would have attained had the early foetal growth-rate been continued. "This suggests", says Scammon, "that the growth of the uterus in the latter foetal months consists of a substratum of typical foetal growth plus a secondary increment due to an extra stimulus furnished by a hormone of placental or possibly ovarian origin." Here is an excellent illustration of how an organ can act as an index registering obscure physico-chemical changes in the internal environment of the embryo. The other organ which undergoes a reduction in size following birth in man is the suprarenal gland, and it also has been investigated by Scammon. But, unlike the uterus, its growth when observed in the foetal period shows no increased intensity towards the time of birth, so that the involution which occurs afterwards by degeneration of the two inner cortical layers decreases its size far below what it would have reached had it gone on growing at the same velocity as before birth.

This leads on to the general problems raised by the growth of the ductless glands in the embryo, problems of the greatest importance in view of the regulating influence which the foetal endocrines probably exercise. The pituitary gland, according to Covell, grows proportionately to the total body-weight in human pre-natal life, i.e. slowly till about the 5th month and more rapidly thereafter. The thyroid also shows no outstanding variations from the normal curve. The growth of the thymus, however, is characterised by high variability, while the pineal gland grows at nearly the same rate as the brain. The results of studies on the weight of these glands, therefore, do not reveal any striking correlations, and they must be supplemented by histological evidence. This will be presented in the section on hormones.

Scammon; Scott; and Scammon & Kittleson have studied the growth of the intestinal tract and the stomach in the human embryo. While this work does not give us any help in evaluating the active absorptive surface during embryonic life, its main conclusions are of interest. Thus the growth of the gastro-intestinal tract follows the law of antero-posterior gradient or direction, for the more cranial portions grow relatively more rapidly in the early part of foetal life, while the successive caudal portions show smaller amounts of growth at the beginning and larger ones later. The number of crypts and glands in the stomach mucosa seems to increase per sq. mm. very regularly during the progress of foetal growth. Watkins' study of the growth of arteries is also interesting, for it shows that the vessels which supply the foetus only have a rate of growth similar to that of the body as a whole, i.e. slow for a short time at first, and then for a long time rapid, while the arteries which supply the placenta as well as the embryonic body have a long period of slow growth followed by a short period of rapid growth. These facts throw a certain light on the metabolic needs of the developing organism. Much valuable information is contained in the papers of Scammon and Armstrong on the foetal growth of the eye, and in that of Noback on the respiratory system, but it cannot be given here.

Davenport has recently considered the growth-curves of man in the light of the work of Scammon and his associates, and Gunther has written on these subjects especially in relation to sex.

2·11. Variability and Correlation

Enough has now been said about the growth of the parts and organs of the whole considered in isolation, and we must consider the relation between the growth-rate and two other factors, namely, variability and correlation. The population of cells in the metazoal embryo may no doubt be compared with the populations of protozoa in cultures, but, whereas the functions of the whole in the latter case are very limited, those of the whole in the former case are highly complex. In other words, one may enquire to what extent there is variability between different embryos of exactly the same fertilisation age. Closely allied to this question is what is, to all intents and purposes, its converse, namely, at what point in development is the correlation coefficient greatest, i.e. at what point is the swing of variation among embryos away from the mean least? It is to be regretted that these enquiries have not been very deeply carried on in embryology, but there are some rather significant observations which need attention.

So far only the mean values for weights and measures of embryos have been under consideration. But obviously no statistical study of these is complete without a consideration of the amount of variability among the individual cases from which the mean value is derived. The variability coefficient is defined as the

$$\frac{\text{standard deviation}}{\text{mean}} \times 100,$$

the standard deviation being a measure of the spread of points around the mean, i.e. a measure of the point upon the frequency-curve where the change takes place between concave to the mean and convex to it. Fig. 22 showing McDowell's points will explain the meaning of this. It has long been known that the variability coefficient decreases with age in man, and it is always stated that it follows the changing growth-rate quite closely, but some confusion has been caused in the past by a doubt as to what manner of representing the growth-rate is being referred to. The fact is, however, that the variability coefficient follows the simple increment curve. Thus, if for absolute growth a sigmoid curve holds good, the greatest daily or monthly increment will occur as we have seen at the middle of the period, and this peak will coincide with a peak in the variability coefficient. This was found to hold in actual fact by Boas & Wissler, by Boas, and by Bowditch, who studied exhaustively the growth of Toronto school-children. The variability coefficient in that case followed exactly the curve of yearly increment and reached an exactly simultaneous maximum at the age of 15 years. The correlation coefficient behaved in the same way. Boas & Wissler explained their results by saying that correlations between measurements in one individual ought naturally to be greater during periods of rapid growth than at other times, because the variations in responsible factors will affect them all to an equal extent. Variability is what governs correlation, so that would also be expected to rise and fall in the same manner. But there is a proviso that must be made here, for Boas & Wissler's use of the term "growth-rate" is not the same as that of Brody, for instance. Boas & Wissler mean by the time of greatest growth-rate the time at which the largest increments are being added on to the organism in unit time, i.e. the half-way point in the curve of the autocatalytic equation. Brody means by the time of greatest growth-rate the time at which the organism is adding on to itself the largest relative increments, i.e. the earliest stages of embryonic life, when the slope of the log. weight/age curve is extremely steep, and the embryo doubles its weight in an exceedingly small lapse of time. The variability coefficient shows, therefore, that it is at any rate true to say that, during the phase when the largest absolute increments are being made, the widest variations from the mean tend to occur. This seems very reasonable, but there is also evidence which shows that, when the period of most rapid growth in Brody's sense is occurring, the variability coefficient is also large.

Other examples are numerous. Buchem found a coefficient of variability of 0·4 for the early stages of the embryo cow and 0·1 later. Edwards found a variability coefficient of 0·1347 for unincubated chick blastoderms, but of 0·1087 for those incubated 24 hours. Jenkinson gives a graph exactly analogous to those of Boas & Wissler computed from Roberts' measurements of English artisans. Jenkinson also worked on the trout embryo (or rather the alevin, for his first point was 2·3 weeks after hatching, by which time the yolk was not completely absorbed). He found that there was a close general agreement between weekly increment and variability coefficient for the first 10 weeks after hatching, true not only for the growth and variability of the body-length but also for some of the parts such as the diameter of the eye, the length of the head, and the length of the caudal fin. Fig. 60, taken from Jenkinson, shows how, although the curves do not run absolutely parallel, they certainly rise and fall together. On the other hand, the correlation coefficients between several pairs of organs show that in many cases—total length and breadth of caudal fin, total length and length of anterior dorsal fin, total length and length of head, head length and eye diameter—there is a significant diminution in value during the time that there is a decrease in growth-rate. Thus, when the growth-rate is highest, the variations between individuals are greatest, but the correlation coefficients between various organs or parts in the same individual are also greatest. It is easy to see why this should be so, but Boas has given a mathematical proof of the relation between these coefficients and the growth-rate. Expressed differently, it could be said that the faster the growth-rate the more proportional the growth but the greater the variation as between individuals.

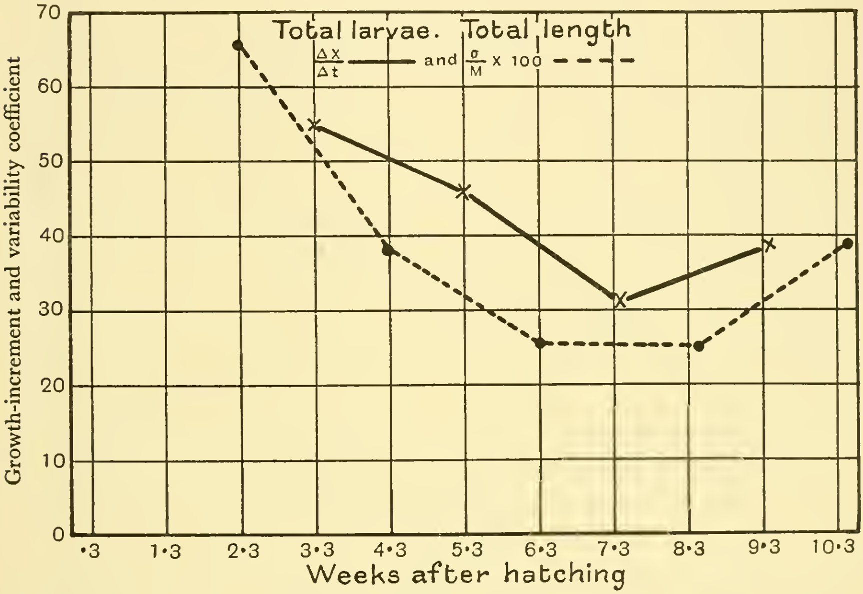

Fig. 60.

Turning now to the early part of the embryonic period, the first complete investigation was that of Fischel, who studied the individual variations between duck embryos at the primitive streak stage. Von Baer had already averred qualitatively as early as 1828 that variability was much more pronounced in the earlier stages than in the later ones. Kupffer & Benecke; Keibel & Abraham; and Assheton afterwards drew special attention to it. Fischel, however, measured the length of the embryos, and found that the older they were the more regularly they agreed together. This led him to conclude that regulating influences came into play during development which brought about a more synchronous course of growth and differentiation, and made the individual variations less and less obvious. Such a standpoint would accord well with frequency-curves such as that of McDowell and his collaborators for the mouse embryo (see Fig. 22), where the range of weights on a given day is 4 or 5 times as large at the beginning of development as it is at the end. His and Levi, working on the development of the chick, came across the same phenomenon.

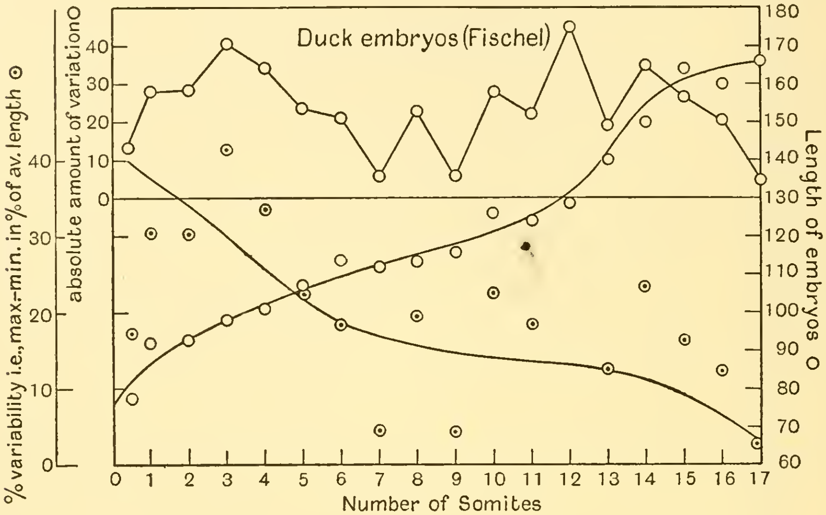

Fig. 61.

Fischel divided the total length of the embryo into a number of constituent lengths, e.g. from the cephalic to the caudal end of the somites and from the extreme cephalic end to the anterior blastopore ("Darmpforte"). He distinguished 13 such lengths, and these he measured in a large number of embryos, judging the age in each case by the number of somites formed. As will be seen from Fig. 61, the limits of variation considered as absolute maxima and minima are much the same when the embryo has 0 somites as when it has 20, but, in view of the increase in total length during this time, it will be seen that the maximum variation is much less important as time goes on. "The relative differences", said Fischel, "are certainly less in the later stages than in the earlier ones." Fischel's measurements of the lengths of the parts all showed the same relation as regards variability, but, though the length of the body is increasing regularly all through this period, the length of the part between the anterior end and the 1st somite remains practically stationary, as does the length of the part between the last somite and the posterior end of the embryo. In other words, the increase in length is entirely due to growth of the middle region in which the somites are being produced.* The size of the individual variations can be large; thus an embryo may be more than 50 per cent. longer than another one of the same stage. Fischel's examination of the work of other authors, such as that of Bonnet on the sheep and of Keibel on the pig, induced him to suppose that very similar effects were seen in mammalian embryos. Philiptschenko carried the question into insect development by investigating an apterygote, *Isotôma cinera*, and found that the older stages showed the greater variability. On the other hand, Zuitin, who has studied the development of *Dixippus morosus* from this point of view, found that, just as in the birds and mammals, the earlier stages were the ones which showed most variations from the mean. Schmalhausen has also considered the question on the basis of the figures he obtained in his studies on the growth of the chick embryo, already referred to. He points out that the early stages in embryonic development are those when the anlages are being formed. At that time, every few hours, as it were, are marked by the start off of one or more parts or organs on the long declining hyperbola which represents their instantaneous growth-rate. Thus a cross-section through an embryo in those early periods would demonstrate, if some method of the future made it possible, a series of growth-rates, some low, appertaining to the more senior organs, some high, appertaining to the more junior ones. Moreover, the mass of each anlage is different for each organ, so that extremely complicated effects will be observed if the weights of the organs are described in percentage of the total body-weight. Thus the earlier the stage the more chance there will be for individual differences in growth-rate to reveal themselves, while in later development these will be equalised, adjusted, and compensated by a process of self-regulation. Schmalhausen does not say, however, what this process of self-regulation is, and its nature, indeed, offers one of the most interesting problems in embryology. We shall refer to it again in the chapter on hormones. Schmalhausen suggests that Philiptschenko's results might be explained by the extremely small time elapsing between the formation of the various anlages in animals with ultra-rapid incubation times such as some insects. Owing to the high instantaneous growth-rates in the earliest stages, a minute difference in time of formation of an anlage or a minute difference in its initial size will make a very large difference between one individual and another.

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* As Levi has shown, embryo size in the somite stage is very similar no matter what the size of the adult bird, but soon the larger animals grow longer. Also the size of the first somites of all sauropsida is between 5000 and 8000 μ^2 surface.

2·12. Explantation and the Growth-promoting Factor

We must now return to consider further the essential nature of the embryonic growth process. Some reference has already been made to Schmalhausen's findings with regard to spurts of growth and the extent to which all organs are affected simultaneously by the growth-impulse. But what is this growth-impulse? What is its essential nature? It is no longer possible to say that we have no idea, for there are a number of very significant observations regarding it arising from the study of tissue culture *in vitro* outside the living body. Ephrussi has brought forward evidence suggesting that growth-rate may be a genetic factor. The study of the effect of temperature on the growth-process has also been fruitful to a certain extent in answering this question, and its results will shortly be described.

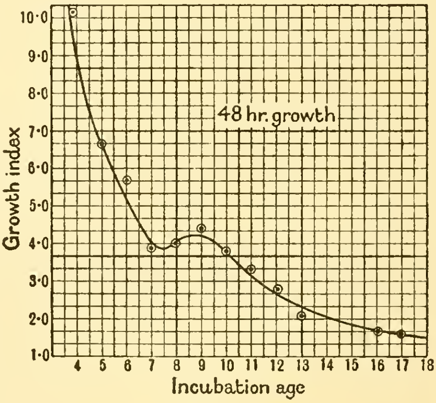

Fig. 62.

In the first place, it is natural to enquire whether the growth-rate found to hold for the entire embryo growing under its normal conditions would hold for parts of it isolated and growing in tissue culture. Would small pieces of it be found to grow in tissue culture proportionately to the growth of the organism as a whole, or is the growth-rate a value absolutely dependent on the intactness of the embryo? This question has found a definite answer in the remarkable work of Cohn & Murray, who, following up a few precursory experiments of Carrel, removed hearts from chick embryos from the 4th day onwards, made cultures of them in adult hen plasma or plasma plus Ringer solution, and determined their rate of growth. This was done by four methods: (*a*) subtracting from the total area of tissue after *n* days of cultivation the area of the central portion, (*b*) dividing the area of growth made (total minus central portion) by the size of the original fragment, (*c*) dividing the area of growth made by the size of the central portion at the time of measurement, and (*d*) dividing the area of growth made by the square root of the area of the central fragment. With these methods and carefully controlled conditions they found that the growth-rate of the cultures of embryo heart fell in an exactly parallel manner to the Minot curve previously found by Murray to hold for the chick. Figs. 62 and 63, taken from Cohn & Murray's paper, show this fall, and may be compared with Fig. 34 which shows the corresponding Minot curve for the whole embryo. The kink in the heart culture curve may perhaps be related to special changes occurring in the organ at the 9th day (see Section 23). It must be remembered that the heart of the chick has been stated by Schmalhausen to grow at the same rate as the whole body, though, as a reference to Table 56 shows, the Schmalhausen *Cvt* is 276 for the heart and 321 for the whole body. However, it would not have been material which organ had been selected by Cohn & Murray, for all organs have growth-rates either descending

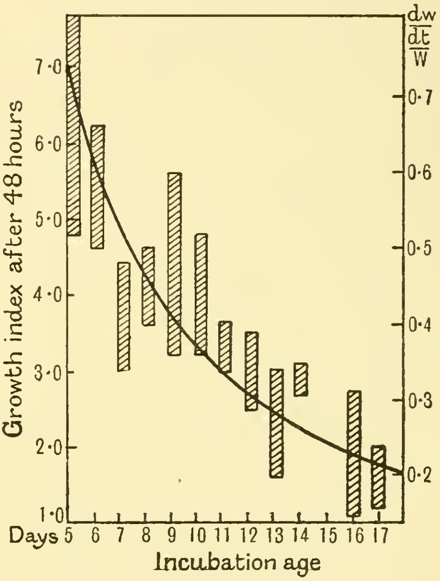

Fig. 63.

in steps (see Fig. 55*a*) as Brody would have it, or in a hyperbola (see Fig. 55*b*) as Schmalhausen would have it. That Cohn & Murray compared their results with the Minot curve does not matter, as all percentage growth-rate curves, whether instantaneous or not, fall towards the abscissa asymptotically. Cohn & Murray's achievement was to demonstrate that the growth-promoting impulse resides to a large extent actually within the cells, and that its fading out can be seen equally well in the decreasing rapidity with which isolated cells will grow in culture as in the decreasing rapidity with which the whole cell-population of the embryo increases in the egg. In other words, functional changes taking place in the organism as a whole are mirrored by similar changes in the individual cells.

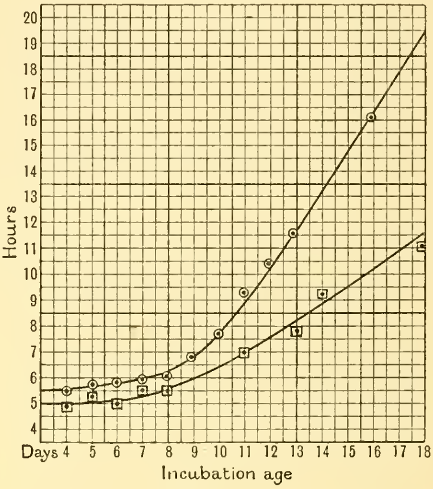

Fig. 64.

Heart-fragments in plasma, ⊙; heart-fragments in plasma + Ringer, ⊡.

Cohn & Murray also determined the latent periods in their chick heart cultures, and were able to show that, as the embryo grew older, so the cells of its heart took longer and longer to accustom themselves to their *in vitro* environment. The latent period was defined as the time intervening between the incubation of the cultures and the first appearance of cells protruding from the peripheral margin. As Fig. 64 shows, this value markedly increases, though not until after the 7th day. Unknown to them, Suzuki had previously found just the same relationship between latent period and age in the chick embryo. His figure (Fig. 65) is a beautiful illustration of their conclusions. Growth-rate and latent period are two strangely associated processes. "When older tissue", as Cohn & Murray put it, "i.e. a heart fragment from a 16-day old chick embryo, is placed in a fresh environment, it does not assume immediately a divisional velocity typical of younger tissue. In our experiments the previous growth-rate was not approached for 36 hours. In other words, a number of cell-divisions (i.e. generations) were required for it to lose the habit of slow growth previously imposed upon it by its organised environment. This happening is an expression of a process of rejuvenescence or dedifferentiation*. The tissue soon assumes a growth-rate determined by its environment, and as long as the environment can be kept relatively stable, that is to say of uniform composition, presumably there will be no further change in divisional velocity."

As in the intact embryo, then, negative acceleration of growth is greatest at the beginning of life. On the other hand, the latent period before growth *in vitro* begins is greatest at the end of embryonic life. It is likely, therefore, that the factors which determine the initiation of growth and those which determine the extent to which it shall take place in a given time are not identical.

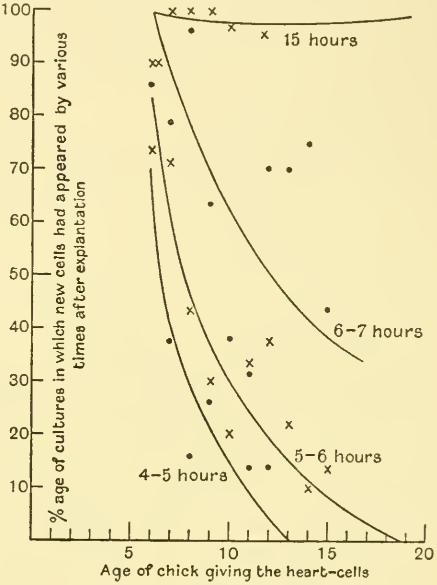

Fig. 65.

Olivo & Slavich and Oda & Kamon, subsequently repeated the experiments of Cohn & Murray and as far as heart fragments were concerned, confirmed them in every particular. Nordmann also confirmed them for liver cells. Oda & Kamon found that the growth-rate in culture was the more rapid the younger the embryo from which the heart had been taken, and the latent period was the shorter. The pulsatile activity of the heart explants was also more hardy the younger the embryo. On the other hand, they did not obtain such clear-cut results with the spleen which to some extent showed a higher *in vitro* growth-rate the older the embryo from which it had been taken. This doubtful point urgently requires re-investigation, for it has become a commonplace of explantation research that growth-rate *in vitro* follows growth-rate *in vivo*. Moreover, Allen has found other differences between organs; thus in saline, embryo heart will not grow at all after the 8th day, intestine after the 11th day, and so on.

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* This does not imply the acquisition of any pluripotence or totipotence.

The experiments of the Cohn & Murray type were precisely the converse?of those of Carrel & Ebeling who found that the growth-rate of a standard 2-year-old strain of fibroblasts was affected by the age of the hen from which the plasma culture medium was derived. The older the donor the lower was the growth-index, the younger the donor the higher, i.e. the faster the explant grew. Thus the growth-promoting factor must be present both in the cell-protoplasm and in the circulating blood plasma or "milieu intérieur". This growth-promoting factor was for long believed to be a hormone or even a vitamine, but it now seems to be much more probably a special collocation of the right nutrient substances, probably protein breakdown-products.

Following upon the pioneer work of Ross Harrison, it was found by Carrel; Carrel & Burrows; Fischer; Ebeling; and Carrel & Ebeling that tissues would grow indefinitely *in vitro* on a medium composed of one part adult plasma and one part embryonic tissue juice. It was then soon found that it was the latter constituent which furnished the necessary factors for cell nutrition and multiplication (Carrel & Ebeling and Carrel). From this point there began an extensive series of researches directed towards the identification of the substance responsible for the effects produced by embryonic tissue juice. Whatever it was, it was not species-specific, for duck fibroblasts were found to grow well in chick embryo extract (Fischer), and *vice versa* (Kaufmann), while rat tissues could be grown in chick embryo extracts (Mottram), and rabbit embryo extracts would stimulate chick tissues (Carrel & Ebeling) or human tissues (Timofeivski & Benevolenskaia).

Results which are interesting in this connection are those of Loisel who substituted duck egg-white for the natural egg-white of the hen's egg and found that the normal development of the intact chick embryo up to the 4th day was not interfered with by these conditions.*

Carrel & Baker examined the fractions obtained from chick embryo juice, and concluded that the growth-promoting factor was associated definitely with the protein part. The precipitated proteins of the embryo juice (acetone, carbon dioxide, alcohols, acetic acid, etc.) never showed a greater growth-promoting power than the original juice, but often nearly as much. Chemical examination showed that the protein in question was a mixture of nucleoprotein and a glucoprotein rather like mucin, but when these were prepared in a pure state, they showed no growth-promoting action in tissue cultures. Carrel & Baker tried a great variety of other substances, especially proteins, such as pure egg-albumen, but always without result. Ether extraction, they found, did not remove the growth-promoting factor from the embryo juice proteins, so that it seemed unlikely that it could be of lipoidal nature.

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* Bouges also, by an injection technique, has replaced the yolk by yolk from other breeds or even species, without interfering with normal development.

Some earlier experiments of Carrel & Ebeling had shown that although pure amino-acids produced a slight stimulation of growth, causing greater cell-migration, they did not produce an increase in the mass of the tissue, as the protein fraction of embryo juice certainly did. This was confirmed by Carrel & Baker, who dialysed the embryo juice in collodion bags of high permeability, or else ultra-filtered it. They found that the dialysed juice did lose a certain amount of its growth-promoting power, but they put this down to denaturation of the proteins, in view of the fact that the amino-acids separated in this way were practically without action on the growth of fibroblasts in culture. Nor was there an enzyme in the juice which produced amino-acids in the dialysing mixture more rapidly than they could diffuse away. It is true that the liquid surrounding the collodion bag and containing the diffusible amino-acids had a slight effect on the increase in area of the explants, the rate being 1·28 times faster than in Tyrode solution, as against 10 times or upward for the protein fraction. This small effect was put down by Carrel & Baker to migration stimulus, and not to increase of mass of the explant. When Carrel & Baker tried to restore the loss of activity of the protein part by adding the amino-acids which had dialysed away, they found it was impossible to do so—a fact which supported the view that the proteins had been denatured by the dialysis. Thinking that the concentration of amino-acids in the untreated juice might be too small to show the presence of the growth-promoting factor, they hydrolysed the juice protein with acids and with trypsin, but the large concentrations of amino-acids so produced proved to be toxic for the cells of the cultures. This toxicity had already been noticed by Burrows & Neymann. Wright also worked with diffusates of embryonic tissue juice and observed a perfectly equal number of mitoses in explants of chick embryo heart, whether the medium used contained embryonic tissue juice itself or the diffusate from it, e.g. 35 ± 5 in the former case and 32 ± 5 in the latter. In another instance the number of mitotic figures was: saline 2, extract 24, diffusate 26. These results were interpreted by Carrel & Baker as being due to a stimulatory action of amino-acids on migration, and they stated that they observed similar phenomena themselves with pure amino-acids, which, however, gave nothing approaching that large extension of area seen in extracts with the protein fraction. All amino-acids, according to Carrel & Baker, stimulate cell-multiplication and migration, without increasing the mass of the tissue, i.e. without causing growth.

In a succeeding paper Carrel & Baker put to the test the suggestion that it was not the proteins as such in the embryonic tissue juice which carried the growth-promoting factor, but rather their larger split-products such as proteoses, broken off from them by the cells of the tissue culture themselves. They found that if tissue juice from embryos was digested with pepsin for 16 or 32 hours, the hydrolysate was rather toxic for the fibroblast explants, but if the digestion was only carried on for 3½ hours, the presence of the growth-promoting factor readily revealed itself, growth proceeding seven times as fast as in Ringer solution. The large protein split-products which were evidently responsible could be obtained just as well from peptic hydrolyses of egg-albumen, and commercial fibrin, the latter especially proving a rich source of growth-promoting activity. Chemical examination of the different fractions of the digests, compared with tissue culture examination, showed that the substances responsible were undoubtedly proteoses. Pepsin digests of rabbit brain were found to be active. Commercial "peptones" were found to vary in activity, according to the proportion of higher split-products contained in them. Tryptic hydrolysis was not so satisfactory. Carrel & Baker suggested that the function of the proteose is to furnish a higher concentration of amino-acids to the cells than could be obtained even from their saturated solutions, and to supply them to the cells in this tightly packed, yet soluble and diffusible form. Carrel & Baker's identification of proteoses as the important substances was confirmed by Fischer & Demuth and by Willmer.

All these facts led Carrel & Baker to conclude that there was no justification for speaking of a "growth-promoting hormone". The growth-promoting factor is probably no more than a right conjunction of nutrient materials and the appropriate capacities for making use of them. The particular position taken by unhydrolysed embryo tissue juice is of course very important from the embryological point of view, and merits much further research.

Still later, Carrel & Baker went on to investigate the growth-promoting activity of digests of pure proteins, fibrin, egg-albumen and edestin. The proteins themselves, of course, had, from the earliest days of tissue culture, been known to be more or less inert (Smyth; Swezy). They now found that pure fibrin, when taken to pieces by pepsin into its proteoses, had as powerful an effect as commercial fibrin, so that the effect was not due to impurities such as blood corpuscles. The split-products of pure egg-albumen, however, did not show so large a growth-promoting action, and Carrel & Baker found that it could be increased by the addition of pure glycine and of pure nucleic acid. It was interesting that digests of vegetable proteins such as edestin and gluten, showed a marked growth-promoting activity. Carrel & Baker also showed that a medium containing a maximum growth-promoting activity could be prepared by digesting calf liver or pituitary with pepsin to the right extent, and that α- and β-proteoses were equally effective.

If now we go back some years we find that in 1921 Carrel & Ebeling found that adult serum strongly inhibited the multiplication of fibroblasts and epithelial cells in tissue culture. As already mentioned, it was not long before they found that this property clearly increased with the age of the animal providing the serum (Carrel & Ebeling). Then in 1927 Carrel & Baker showed that this action was due to changes with age in both the lipoid and protein fractions of the adult serum, and that in all probability it was related to the increase of antitrypsin: a significant finding if the hydrolysis of the proteose by the explanted cells is the most fundamental mechanism involved. Carrel & Baker showed that the growth of fibroblasts was 1·56 times as good in the young as in the old serum.

Brody, who was interested in this property of serum as an index of senescence, constructed a chart showing the decline of its growth-promoting power with age, and this is reproduced as Fig. 66. Duration of life of fibroblasts grown in adult serum, plotted against age of fowl giving the serum, declines with a k (instantaneous per cent. growth-rate) of 0·18 and halves itself in 3·9 years. The data from which this graph is derived were given to Brody by Carrel.

After these demonstrations of the nature of the growth-promoting factor, the assertions of other workers that a hormone is in reality concerned do not seem very convincing. The paper of Heaton may be consulted for experiments done from this point of view. The fact that tissue cultures of very early chick embryos are not successful and the fact that observers are agreed on the necessity of keeping the cells in numbers for successful cultivation led Wright to investigate how it is that in nature the early stages of the chick are ever successfully passed through. Yolk from the hen's egg proves itself inactive when added to tissue cultures, probably because, as Carrel & Baker showed, yolk-lipoids inhibit growth in explants, but Wright found that on dialysing the yolk a substance passed through the membrane which behaved very similarly to the dialysates from embryo tissue juice. Thus in one experiment where the yolk diffusate from 7–8-day eggs was tested on heart fragments of from 10–11 days' incubation, the number of mitoses in the treated explant was from 114 to 127, while in the control it was only 14. Willmer has discussed these observations in an interesting review.

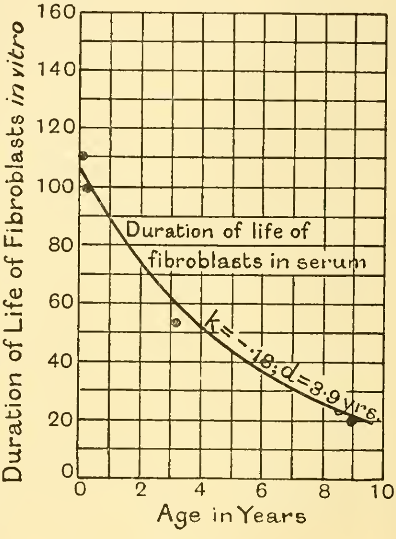

Fig. 66.

Fischer has elaborated a theory of "desmones" or mutually stimulating substances, and Burrows has introduced the terms "archusia" and "ergusia" for similar ideas. It is not possible to review the work of these authors here. Burrows & Jorstad did, however, point out that the growth of embryonic cells in tissue culture depends on sufficient crowding and a certain stagnation of the medium. For reasons not at all obvious to the biochemist, Burrows & Jorstad identified archusia with vitamine B and ergusia with vitamine A. Still less satisfactory is the work of Carnot, of Roulet and of Carnot & Terriss who affirm, on very slender evidence, that wounds of metazoal animals heal much quicker when treated with embryo extract than when untreated. Carnot & Carnot have also maintained that injection of foetal extracts into unilaterally nephrectomised animals causes a much increased compensatory hypertrophy of the remaining kidney.

Carnot has also studied the effect of chick and rabbit embryo extracts on the growth of tadpoles, using glycerol extracts or the powdered dry substance. There was no influence on metamorphosis, but a great increase of growth, the experimental tadpoles being two or three times as big as the controls. This recalls the older observations of Springer who maintained that the eggs of *Arbacia punctulata* and *Asterias forbesii* contained a substance inhibitory to the growth of other eggs of the same species. The retardation was noted in the early rather than the late embryos, and there was a marked tendency for the eggs to stop development when they arrived at the blastula stage. Subsequent work by Peebles indicates that a growth-promoting factor (perhaps a proteose) may also be found in echinoderm embryos. These observations are not very convincing and perhaps the subject might be re-examined with advantage.

Experiments which may, when extended further, throw some light on these questions have been made by Skubiszevski, who transplanted chick embryo tissues into adult hens. The complete failures were more or less numerous at all stages of development, and so probably represented errors of technique, but the conspicuous successes gave a curve which was markedly peaked at the 3rd day of development. The grafts grew well without antagonism for as much as 72 days as mesodermal connective tissue, but in three cases (out of 178) they resembled a sarcoma at the end of that time. This 3rd day peak may be an important clue. Precisely similar work on grafts was done by Uhlenluth, who transplanted the eyes and skin of larval salamanders into individuals of various ages. These transplanted organs attained maturity not at the time when their original possessor took on adult characteristics, but at the time when their new possessor metamorphosed. They fell into step, as it were, with their new environment. The hormones and growth-promoting substances which regulate such changes are still imperfectly understood, but Babak; Gudernatsch; Swingle; Huxley & Hogben and many other workers, have fully unveiled the importance which thyroxin has in amphibian metamorphosis.

Another way of studying the essential nature of the growth-impulse might be to plot the viability for *in vitro* tissue culture of individual cells from embryos killed at a definite time. Perhaps it would be found that the younger the embryo, the longer would its component cells remain viable after its demise as a complete whole. Thus Bianchini & Evangelisti found that in guinea-pig and rabbit foetuses the cells of tissues were cultivatable as long as 78 hours after the death of the whole body. And Bucciante, who incubated hen's eggs for 6–12 days and then placed them at 0 or 15°, found that *in vitro* cultivation was still possible for as long as 25 days afterwards in the case of epithelial cells, though leucocytes and liver-cells only retained viability for 3 or 4 days. Cells of other tissues occupied various intermediate positions.

2·13. Incubation Time and Gestation Time

So far, the total time taken in embryonic growth has not been considered at all. It was indeed said, when the work of Donaldson, Dunn & Watson was being considered, that the act of birth or hatching seems sometimes to have little influence on the course of growth, but as Brody and Schmalhausen have shown, it more commonly affects profoundly the rate of increase of size. And it does set a term to the embryonic period (I make no distinction between embryonic and foetal period, as is the custom with some authors), forming at least a convenient index to show whether the growth of the embryo is being accelerated or retarded by external influences. It is therefore important to know the normal incubation or gestation time for as many animals as possible. These are collected together in Tables 60, 63 and 64. It can easily be seen that there is some difference between the estimates of gestation and incubation times in the same animal by different workers, but these discrepancies can only be cleared up by more extended observations.

When we come to consider, however, the nature of the law which must presumably govern the length of embryonic life we meet with a remarkable degree of obscurity. Roughly speaking, the common-sense rule that the larger an animal is, the longer its embryonic life must be, is borne out by the figures. Thus in Table 63, where the weight of the adult mammal is seen to vary between 0·014 kilo and nearly 4000·0 kilos and the gestation time between 21 and 600 days, the larger mammals have the longest gestation times, although there are several cases where animals of the same weight have different gestation times (e.g. the pig and the deer) and animals of the same gestation time reach, when full-grown, very different weights (e.g. the antelope and the hippopotamus). However, if the weight when adult is plotted against the gestation time on double-log. paper, a straight-line relation is obtained, individual points not lying very far from the mean. The only reason for using double-log. paper here is the convenience of getting all the data on to the same graph. The line does not meet the co-ordinates at the zero point; it cuts the time scale, no matter what units are taken. This means that no matter how small the mammal, an appreciable time has to be taken in development, and in the case of a mammal as small as a gnat, a surprisingly long gestation time would be observed. Thus the mouse, which is 259,000 times as small as an elephant, does not have an incubation period 259,000 times as

Table 60. *Przibram's figures.*

Animal	Gestation time (days)	Birth-weight (gm.)
Chimpanzee (*Troglodytes niger*)	260	1,000
Macacus (*Macacus sinicus*)	160–210	480
Mandrill (*Cynocephalus papio*)	177–210	480
Uistiti (*Callithrix jaccus*)	84	33
Lemur (*Lemur catta*)	144	240
Lion (*Felis leo*)	105	1,000
Puma (*Felis concolor*)	92	500
Ermine (*Mustela erminea*)	74	25
Wolf (*Canis lupus*)	61	250
Bear (*Ursus arctus*)	240	1,500
Seal (*Phoca*)	350	10,000
German marmot (*Cricetus frumentarius*)	21	25
Mouse (*Mus musculus*)	21	1·6
Rat (*Epimys rattus*)	21	5
Rabbit (*Lepus cuniculus*)	30	70
Guinea-pig (*Cavia cobaia*)	63	120
Cow (*Bos taurus*)	285	37,000
Sheep (*Ovis aries*)	150	4,000
Goat (*Capra hircus*)	150	2,800
Stag (*Cervus elaphus*)	240–270	24,000
Roedeer (*Cervus capreolus*)	280–300	3,000
Eland (*Alces palmatus*)	240–270	60,000
Camel (*Camelus dromedarius*)	360–400	80,000
Moschus (*Moschus moschiferus*)	160	220
Pig (*Sus scrofa*)	120	1,700
Hippopotamus (*Hippopotamus amphibius*)	210–250	50,000
Rhinoceros (*Rhinoceros unicornis*)	510–550	50,000
Horse (*Equus caballus*)	330–350	40,000–70,000
Donkey (*Equus asinus*)	360–380	20,000
Elephant (*Elephas indicus*)	615–628	240,000
Kangaroo-rat (*Hypsiprymnus cuniculus*)	8½	0·5
Opossum (*Didelphys virginiana*)	8	0·5

short, but only 31·6 times as short. A mammal as small as a gnat, therefore, would probably have a gestation period of 8 or 9 days. The explanation of this must lie in the time requirement of differentiation.

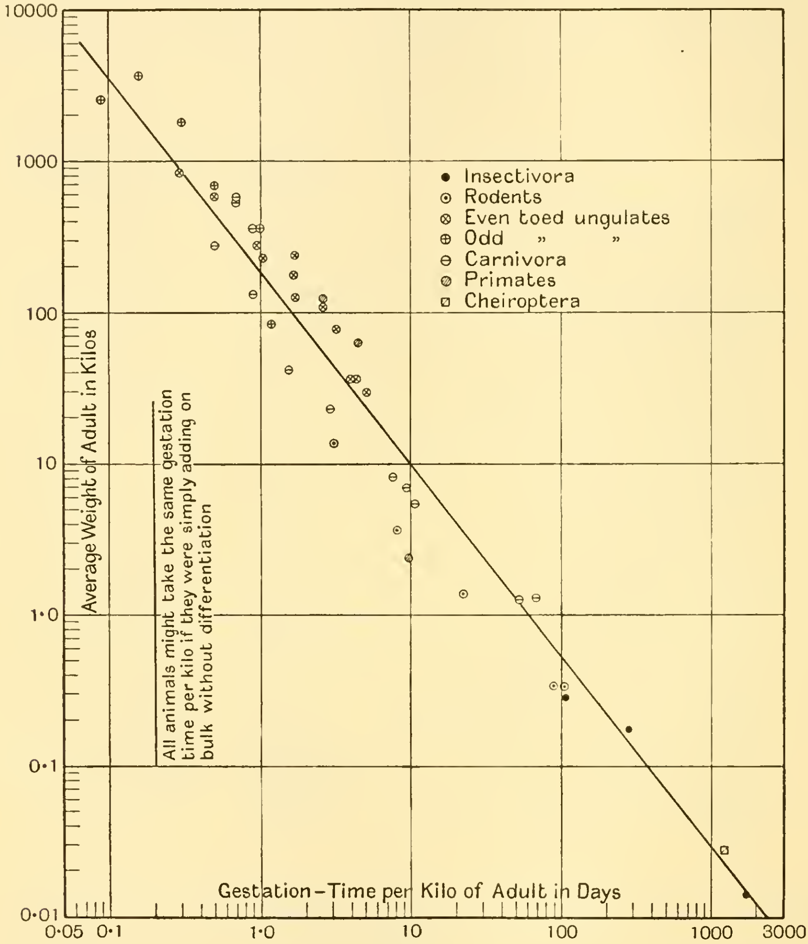

Fig. 67.

Another way of looking at the data appears in column 10 of Table 63 and in Fig. 67. In order to eliminate the factor of weight, the gestation time in days per kilo of adult animal is calculated, and this

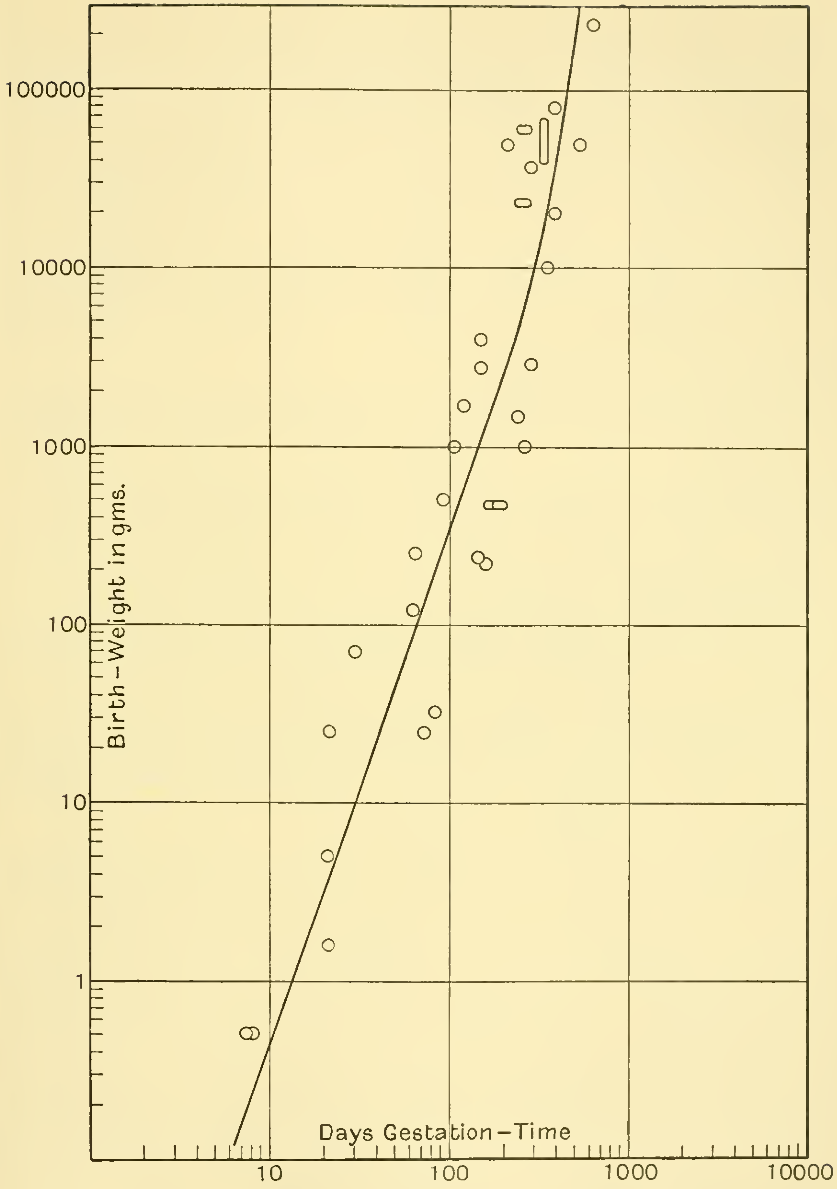
100000
10000
1000
100
10
1
Birth-Weight in gms.
Days Gestation-Time
10
100
1000
10000

Fig. 68.

is then plotted on double-log. paper against the weight of the adult animal in kilos. Evidently the relation is also linear in this case, but the interesting thing is that the smallest animals take far the longest time to construct unit weight. Thus the mouse performs the feat of producing a kilo of mice in 1790 days while the elephant produces a kilo of elephant in 0·16 day. This must be due to the fact that contained in 1 kilo of mouse there is a great deal more organisation and differentiation than in 1 kilo of elephant, in other words that the degree of heterogeneity is greater. Whether all mammals can make unit quantity of differentiation in the same time is a question one would like to have answered, but which seems to be at the present time unanswerable.

So far, we have only considered the relation between adult weight and gestation time. It would obviously be better to use birth-weights for this purpose, but unfortunately only a few are known (see Table 60 taken from Przibram). Nevertheless when the birth-weight is plotted against the gestation time on double-log. paper, a straight-line relation is found, except for a slight deviation in the case of the heaviest animals; this is shown in Fig. 68. The scattering of the points is evidently considerable, but we may say that there is some law which ensures that certain limits shall be held to. Thus if an animal proposes to weigh 100 gm. at birth it must resign itself to an incubation period of between 40 and 150 days, while if it is to weigh 1 gm. it may be between 10 and 30 days *in utero*. Within these wide limits individual species evidently have the power of making drastic shortenings or lengthenings.

Thus gestation time alone may not be a very fundamental constant. In the first place there are great differences in degree of development at birth between such animals as the pig on the one hand and the rat on the other, the former being born almost ready to assume complete motor control of its musculature, the latter by no means ready to do so; the former able to see, the latter blind; the former covered with hair, the latter hairless. Any relation between weight and gestation time can therefore only be approximate, and the law governing it must be, as it were, elastic. Again the difference between polytocous and monotocous animals will make itself felt, and the large differences between the relative weights of new-born and mother. The following table (Table 61), which has been constructed from Franck's information, shows how large these are:

Table 61.

	Total mass of foetal tissue formed : Weight of mother	Weight of one new-born : Weight of mother
	1 : x	1 : x
	x	x
Man	19·1	19·1
Horse	14·6	14·6
Cow	15·5	15·5
Sheep	12·9	12·9
Dog	7·5	23·5
Cat	8·9	37·3
Rabbit	8·5	43·1
Pig	8·2	98·0

And there is also the consideration that gestation time in some animals must be arranged to suit the grazing season. This factor would probably account for a good many of the divergences of species from the line shown in Fig. 68.

Again, within the individual species, birth can apparently be shifted to some extent backwards and forwards. Bluhm's work shows that the opening of the eye, the appearance of the ears, and other marks of increasing differentiation in the mouse, occur at a fixed time after conception, so that the smaller the birth-weight the longer the time between birth and the appearance of the mark in question. This relation is illustrated by Fig. 69.

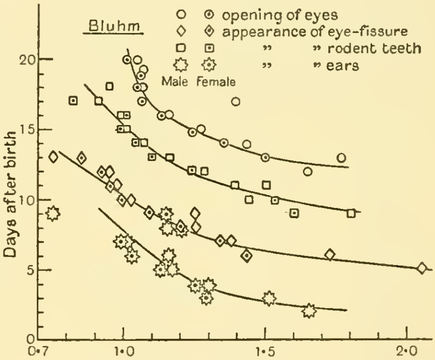

Fig. 69.

What governs the incubation times of birds? The problem has been much discussed, but by far the best treatment of it in the literature at present is the book of Bergtold. Of the 19,000 species of birds known, we have information concerning the incubation periods of 625, and although most of the facts are given in Table 62 Bergtold's book must be consulted for the full material. The length of the incubation period varies more or less with the size of the bird.

Féré long ago pointed out that the smaller the egg the smaller the incubation time; thus:

	Days	Weight of egg in gm.	Ratio
Duck	25	73·9	1 : 0·84
Hen	21	60·18	1 : 0·815

but this strict relation does not hold for many birds and is even more elastic than with mammals. Thus the swift and the raven have the same incubation period in spite of their different sizes, while the kiwi and the hen are very similar in size but have quite different incubation periods. The lapwing, again, though smaller than the woodcock, undoubtedly has a longer incubation period. Nevertheless, when a broad view of the whole subject is taken, and the incubation time is plotted against the adult weight on double-log. paper, a definite trend does appear (see Fig. 70) and the same kind of picture is obtained when the incubation time is plotted against the egg-weight (see Fig. 71). The most interesting thing to notice is the slope of these two lines, which is in both cases much less considerable than in the mammalian graph of Fig. 68. In other words, if the weight of any mammal is multiplied one thousand times, the gestation period will be prolonged by about ten times, but if

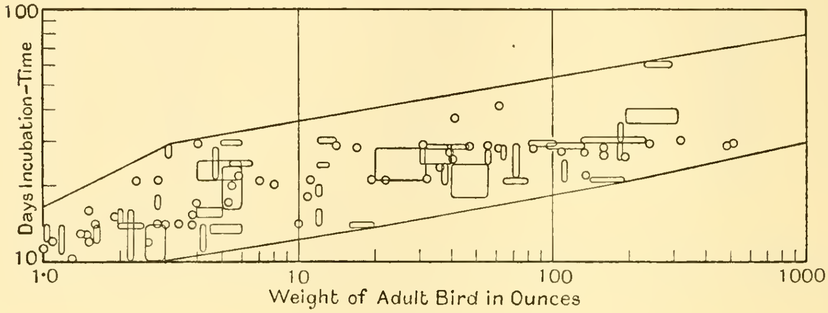

Fig. 70.

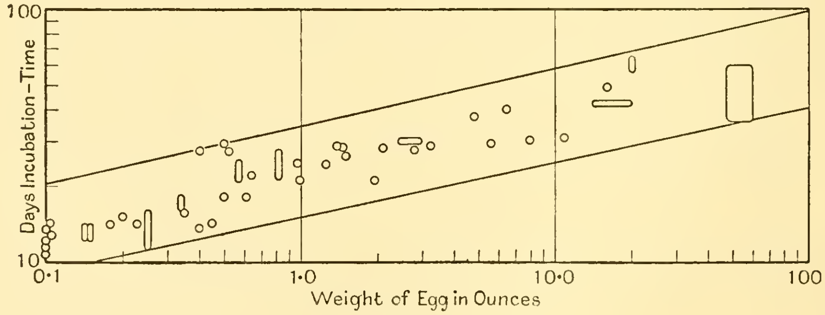

Fig. 71.

the weight of a bird is multiplied one thousand times, the incubation period will only be prolonged about four times. Similarly if the egg-weight is increased by one thousand times, the incubation time is only prolonged four times. No doubt this does not take us very far, but it is always a step forward to have all the information on one graph.

Table 62. *Bergtold's figures.*

Order	Species	Incubation time (days)	Weight of adult bird (oz.)	Weight of egg (oz.)
Struthionidae	Ostrich	36–60	4000	48–60
Dromaeidae	Emu*	56–63	—	20
Spheniscidae	Emperor penguin	49	1440	16
	Adelie penguin	37	42	4–5
Diomedeidae	Albatross	60	224–288	—
Phaethontidea	Yellow-billed tropic bird ...	28	14	1·4
Apterygidae	Kiwi	42	60–65	14–20
Pelicanidae	Pelican	28	512	—
	White pelican	29	240	—
Ardeidae	Great blue heron	28	96–128	—
	Common heron	25–28	64	—
	Loon	29	—	5·7
	Black-crowned night heron ...	24	—	1·2
Ibididae	Wood ibis	21	144–192	—
Anatidae	Domestic duck	27	128	—
	Shoveller duck	28	17	—
	Mallard duck	26–28	—	2·8
	Pekin duck	30	—	2–3
	Grey wild goose	28	160	—
	Greater snow goose	29	80–104	—
	Canadian goose	28	128–224	—
	Domestic goose	28	—	6
	Whistling swan	35–40	192–304	—
Cathartidae	Californian vulture	29–31	320	11
Falconidae	Gyrfalcon	28	84	—
	Prairie falcon	21–28	22–72	—
	Western sparrow-hawk ...	21–28	5	—
	European sparrow-hawk ...	29–30	5–6	0·5
	Eastern sparrow-hawk ...	29–30	4	—
	Honey-eater	21	32	—
	American goshawk	28	47	—
	Western red-tailed hawk ...	28	48–64	2
	Buzzard	28	32–40	—
	Red-shouldered hawk ...	28	32–48	—
	Swainson's hawk	25–28	26–56	—
	American rough-legged hawk	28	30–33	—
	Golden eagle	25–35	160–184	—
	Bald eagle	28–36	128–192	—
Cracidae	Globose currasow	28	114	8
Megapodidae	Mallee fowl	38–41	—	6·5
Phasianidae	Domestic turkey	28	—	3·2
	Bobwhite	24	5·5–6·5	—
	Scaled quail	21	7–8	—

* Haswell gives 84 days and 21 oz. for this bird.

Table 62. *Bergtold's figures* (cont.).

Order	Species	Incubation time (days)	Weight of adult bird (oz.)	Weight of egg (oz.)
Phasianidae	Grey partridge	24	12–13	—
	Capercailzie	26	184	—
	Dusky grouse	18–24	40–56	—
	Ruffed grouse	24–28	18–40	—
	Sage grouse	22	128	—
	Wild turkey	28	160–288	—
	Guinea-fowl	25–28	56	1·4
	Ring-neck pheasant	24	36	1·2
	Golden pheasant	21	20–24	1·0
	Silver pheasant	26	—	1·5
	Reeves' pheasant	24	—	0·98
	Domestic hen	21	64–80	1·9–2·1
Rallidae	American coot	14	16–20	—
Otididae	Great bustard	28	480	—
Charadriidae	European woodcock	20	8–27	—
	American woodcock	20	5–9	—
	Common snipe	20	3–8	—
	Spotted sandpiper	15–16	1·53	—
	Curlew	30	12–14	—
	Killdeer	26–28	3·1	0·4
	Mountain plover	27	—	0·5
Laridae	Lesser tern	14–16	2·0	—
	Common tern	21–23	—	0·6
Columbidae	Band-tailed pigeon	18–20	12	—
	Domestic pigeon	14–18	10	0·5
	Passenger pigeon	14–16	12	—
	Mourning dove	13–14	4·5–6·0	0·4
Cuculidae	Roadrunner	18	11	—
Psittacidae	Cockatoo parrakeet	21	2·9	—
	White cockatoo	21	21	—
	Rose-breasted cockatoo	21	19	—
	Blue and yellow macaw	20–25	37	—
Alcedinidae	Belted kingfisher	16–24	5–6	0·45
Strigidae	Long-eared owl	21	11	0·8
	Barred owl	21–28	20–32	—
	Screech owl	21–25	4–6	0·6
	Eagle owl	21–24	112	—
	Burrowing owl	21–28	6	—
Caprimulgidae	Western nighthawk	16–18	2·7	0·35
Trochilidae	Broad-tailed humming-bird	—	0·1	0·02
Picidae	Hairy woodpecker	14	3	—
	Downy woodpecker	12	1·5	—
	Williamson's sapsucker	14	1·6	—
	Red-headed woodpecker	14	2·8	—
	Lewis's woodpecker	14	3·8	—
	Flicker	11–14	4·3	0·25
Tyrannidae	Kingbird	12–14	1·6	0·15
	Arkansas kingbird	12–14	1·6	0·14
	Say's phoebe	12	0·9	—
Alaudidae	Horned lark	11–14	1·2	—
Turdidae	Western robin	14	3·3	0·23
	Eastern robin	14	—	0·06

Table 62. *Bergtold's figures* (cont.).

Order	Species	Incubation time (days)	Weight of adult bird (oz.)	Weight of egg (oz.)
Mimidae	Catbird	12–13	1·4	0·06
Bombycilidae	Bohemian waxwing	10–16	2·2	—
Troglodytidae	Western house-wren	10	0·5	—
Laniidae	White-rumped shrike	15	2·0	—
Hirundinidae	Barn swallow	11–13	—	0·05
	Tree swallow	14	—	0·06
	Cliff swallow	12–14	—	0·07
Vireonidae	Warbling vireo	12	0·5	—
	Red-eyed vireo	12–14	—	0·07
Sittidae	Rocky mountain nuthatch	13–14	0·65	—
	Pigmy nuthatch	12	0·38	—
Corvidae	Long-tailed chickadee	11–14	0·4	—
	Magpie	17	5·3	—
	Long-crested jay	17	4·0	—
	Crow	16–18	—	0·6
Miniotiltidae	Yellow warbler	10	0·35	0·04
	Myrtle warbler	12–13	0·45	—
	Ovenbird	12	—	0·09
	Redstart	12	—	0·05
Icteridae	Western meadow lark	15	4	0·2
	Brewer's blackbird	14	2·6	0·18
	Red-winged blackbird	10–14	1·6–3·0	—
	Rusty blackbird	14	2–2·5	—
	Bronzed grackle	13–16	3·8	—
Tanagridae	Western tanager	12	1·1	—
Fringillidae	House finch	14	0·66	0·08
	Arkansas goldfinch	12–14	0·47	—
	Pine siskin	13–14	0·43	—
	English sparrow	12–14	1·05	0·09
	Western vesper sparrow	11–13	0·9	0·09
	Lark sparrow	12	0·95	0·07
	Red-backed junco	11–12	0·7	—
	Spurred towhee	12–13	1·5	—
	Black-headed grosbeak	10	1·3	0·10
	Chipping sparrow	10–12	—	0·05
	Lazuli bunting	12	—	0·07

Gurney's theory was that incubation time depended on longevity. The view of Gadow—at first sight more acceptable—was that the developmental period as a whole was uniform and the longer the egg period the shorter the nest period. Yet this simply raises another question, and while it is more difficult to determine the total developmental period than the incubation period alone, the difficulty of relating the preparatory period, whatever it is, to the causal factor, still remains. No doubt the differences between nidicolous and nidifugous birds are removed by this means. Claus' theory was that incubation period depended on egg-size, i.e. egg-weight, but this as we have seen is only true within wide limits, for the ostrich and the kiwi have equal incubation lengths, yet the ostrich's egg weighs $3\frac{3}{4}$ lb. while that of the kiwi weighs less than 1 lb. It is natural that if incubation time depends to some extent directly upon egg-weight, it should depend upon bird body-weight; for as Huxley has shown, the egg-weight varies closely with the body-weight, though the eggs of large birds are not as large as they should be in proportion. Lastly, Pycraft had a theory that incubation time depended on yolk-weight, but as neither he nor anyone else accumulated any data with which to test the hypothesis, and as it is not in any case a very attractive one, it may be dismissed at once.

In Bergtold's view the body-temperature of the parent bird is the important factor. It is likely *a priori* that the larger the bird the lower its body-temperature, and a degree or two may make a big difference. Bergtold gives in his book a long list of bird temperatures and it certainly seems that the smaller the bird the higher the reading, but unfortunately the data are as yet too few for it to appear whether the exceptions noted above as destroying other theories are abolished on this one. Bergtold's theory is complicated by various taxonomic considerations, in which he supposes, following Sutherland, that the higher a bird is taxonomically, the higher its temperature. As the smaller birds (and mammals) are believed to be the most recent palaeontologically, this may well be the case.

In favour of Bergtold's view are the experiments of Heinroth who reported that the eggs of the Egyptian goose hatch in 28 days under a common hen and in 30 days under a Muscovy duck. It is known (see Fig. 83 *a*) that within narrow limits, the speed of embryonic development in ordinary hen's eggs can be controlled by temperature regulation. "The diminishing size of birds," says Bergtold, "accelerated the metabolic rate, elevated the body-temperature, and so shortened the incubation period." According to Bergtold the scanty data of reptilian incubation times support his temperature theory.

Returning now to the comparison between mammals and birds which was raised by Figs. 68 and 71 in which the slope of their weight/incubation-time lines was seen to be different, it is interesting to plot the two on the same graph, as is done in Fig. 72. It can now be seen that not only are the slopes different, but the absolute values are also different, so that on the whole it takes less

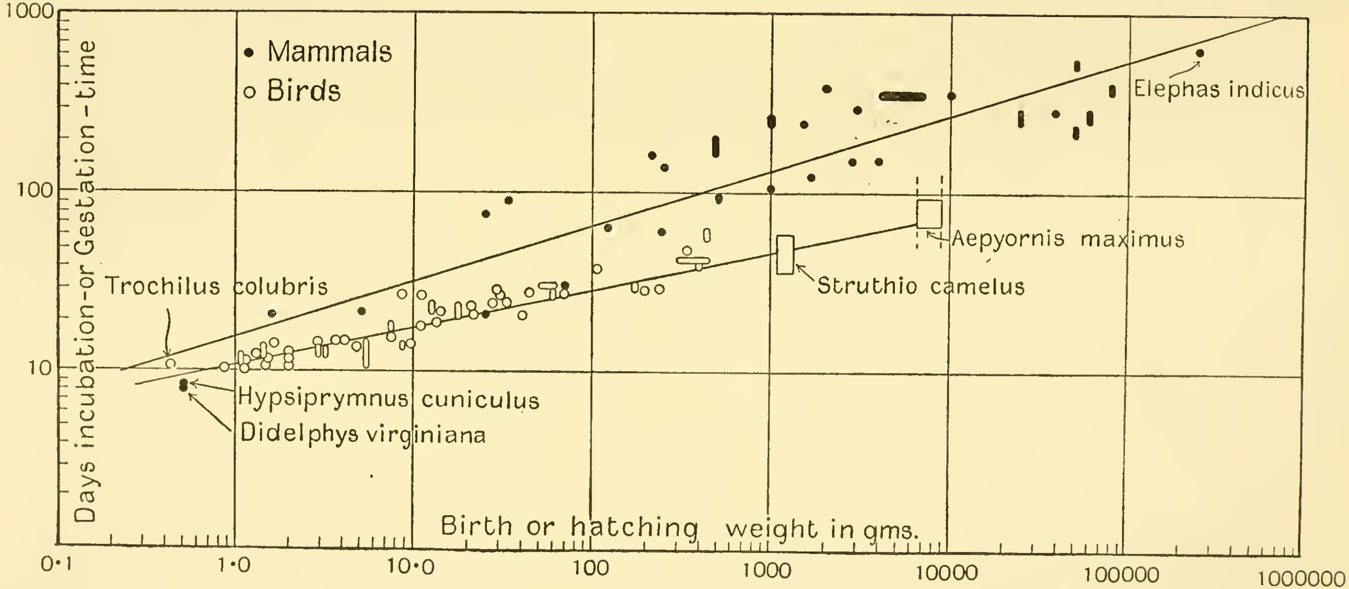
• Mammals
○ Birds
Days incubation-or Gestation-time
Birth or hatching weight in gms.
1000
100
10
0·1
1·0
10·0
100
1000
10000
100000
1000000
Elephas indicus
Aepyornis maximus
Struthio camelus
Trochilus colubris
Hypsiprymnus cuniculus
Didelphys virginiana

Fig. 72.

time to make an equivalent birth-weight of bird than of mammal. (The hatching-weights are here obtained by taking 75 per cent. of the egg-weight in grams, the remaining 25 per cent. being of course divided between shell-weight, weight of membranes left behind, weight of water-vapour evaporated during incubation, and weight of material combusted in the same period.) It is also evident that the largest bird is, as regards birth-weight, 250 times as small as the largest mammal. We are thus left with the following three considerations (which apply wholly to birth-weight):

(1) Although there are mammals as small as the smallest birds, there are no birds as large as the largest mammals. In fact the largest bird is only a little larger than the half-way point on the mammalian line.

(2) The time required to make a given weight of bird is always less than that required to make a given weight of mammal, as may be roughly expressed by the following table:

	Birds	Mammals
Birth- or hatching-weight (gm.)	Incubation time (days)	Gestation time (days)
100,000	—	600
10,000	—	260
1,000	45	150
100	30	55
10	17	32
1	11	14

(3) The prolongation of the incubation time caused by raising the hatching-weight a given amount is not so considerable as the prolongation of the gestation time caused by raising the birth-weight by the same amount.

The bird is therefore much more rapid than the mammal in its development, and one may well ask whether this is not an adaptation to life within the egg. In Section 9 and in the Epilegomena the conception of the "cleidoic" egg will be developed, but without forestalling those discussions, it may be said here that eggs such as those of reptiles, birds and insects, with their isolation from their terrestrial environment, quite unlike the close dependence of many aquatic eggs upon the sea, are closed systems, characterised, as it seems, by a definite type of metabolism in which protein breakdown is suppressed and uric acid takes the place of urea and ammonia as nitrogenous waste products. If, then, there are serious problems confronting animals which make their embryos develop in closed boxes, especially with regard to the disposal of incombustible waste, is it not possible that their incubation time would naturally tend to be shorter than that of beings such as mammals which can conveniently excrete their embryonic waste products through the maternal kidneys? It is perhaps justifiable, therefore, to see in Fig. 72 the results of the closed-box system, development inside it being adaptively hastened. It would be very interesting to have parallel sets of data for insects and reptiles, and one might predict that they also would take relatively shorter times than the mammals, but so far I have not succeeded in finding any data from which graphs could be constructed.

As for the comparatively mean size of the largest bird at hatching compared with that of the largest mammal at birth, it has been probably more than once suggested that eggs above a certain size would begin to suffer from prohibitive mechanical difficulties. An egg large enough to produce a bird as big as an elephant at birth would require, either internal struts, which would be impracticable, or else an extremely thick shell (see Friese's work, p. 239) which would raise great difficulties with respect to gaseous exchange. It is likely, therefore, that 100 days is the extreme limit to which oviparous animals can prolong their incubation time (without hibernating), as against the 600 or more which are possible to mammals. Is this connected with the extinction of the *Aepyornis*?

It might well be asked at this point how it was that the extinct reptiles attained their prodigious size if they were oviparous, and the answer seems to be that for the most part they were not. Some form of ovoviviparity was common, judging from the numerous finds of small skeletons within the abdominal areas of the larger ones. Whether these were really embryos or perhaps rather remnants of undigested food is not yet, and probably never will be, certain, but the question has been discussed by Fraas; Liepmann; van Straalen, and others and the general opinion is that they should be regarded as embryos.

We may conclude that the relatively rapid development of birds is an adaptation to cleidoic life, perhaps associated with the high temperatures of birds.

It is interesting that hibernation of embryos is not unknown. The best known case of this is probably the silkworm, the embryo of which spends about 8½ months in a more or less quiescent state, not advancing to any extent with its development. Dendy reported in

1898 that the embryo of *Sphenodon*, the tuatara lizard, had an incubation period of 13 months, of which something like 9 were spent in a hibernatory state. Boulenger observed much the same thing in the case of the European pond-tortoise, *Emys orbicularis*, which has an apparent incubation period of no less than 23 months. Still more extraordinary is the case of some mammals which possess the power, according to Reinhardt and Prell, of hibernating in the embryonic, partly-completed, state (mole, roedeer, bear, badger, pinemarten, and stonemarten). And as for the insects, Regen has shown that the eggs of a locust, *Thamnotrizon apterus*, laid in September, hibernate *two* or *three* winters and finally hatch out in March. Hibernation, indeed, is very common among insect embryos, the mosquito for instance (*Aedes flavescens*) occupies 7 months in its egg (Hearle).

Table 63. *Gestation times of mammals.*

Species	Milne-Edwards (days)	Schenk (days)	Harmer (days)	Przibram (days)	Hinton (days)	Vignes (days)	Millais; Seton and Briffault (days)	Briffault, average weight of adults (kilos)	Gestation time per kilo (days)
Marsupials									
Opossum	—	—	—	8	—	—	13	—	—
Kangaroo-rat	—	—	—	8·5	—	—	—	—	—
Small kangaroo	—	—	—	—	—	—	38	—	—
Large kangaroo	—	—	—	39	—	—	40	—	—
Insectivora									
Mole	—	—	—	—	—	29	30	0·283	105·8
Ant-eater	—	—	—	190	—	—	—	—	—
Hedgehog	49	49	—	—	—	42	49	0·175	280·0
Cheiroptera									
Bat	—	—	—	—	—	34	36	0·028	1280·0
Cetacea									
Whale	—	—	365	360	315	—	—	—	—
Dolphin	—	—	—	300–360	—	—	—	—	—
Rodents									
Mouse	21	21	—	21–23	—	24	25	0·014	1790·0
Rat	35	35	—	21	—	30	35	0·340	103·0
Rabbit	28	28	—	30	—	29	30	1·360	22·0
Hare	—	28	—	28–35	—	29	30	3·640	8·2
Squirrel	28	—	—	—	—	28	30	0·340	88·0
Beaver	—	119	—	42	—	119	42	13·600	3·1
Marmot	—	35	—	42	—	35	—	—	—
German marmot	—	28	—	21	—	—	—	—	—
Guinea-pig	—	—	—	63	—	64	—	—	—
Even-toed Ungulates									
Sheep	147	147	—	150	—	150	150	29·450	5·1
Chamois	154	154	—	—	—	—	140	36·250	3·9
Gazelle	154	154	—	—	—	—	150	36·250	4·2
Red deer	266	168	—	240–270	—	280	245	77·000	3·2
Gnu	—	—	—	—	—	—	238	226·200	1·05
Reindeer	—	—	—	280–300	—	232	210	126·500	1·7

Table 63. *Gestation times of mammals* (cont.).

Species	Milne-Edwards (days)	Schenk (days)	Harmer (days)	Przibram (days)	Hinton (days)	Vignes (days)	Millais; Seton and Briffault (days)	Briffault, average weight of adults (kilos)	Gestation time per kilo (days)
Even-toed Ungulates									
Elk	—	—	—	240–270	—	266	260	272·000	0·96
Llama	168	168	—	330–360	—	—	300	113·000	2·65
Camel	365	—	—	360–400	—	328	300	181·000	1·7
Antelope	—	—	—	180–210	—	—	—	—	—
Giraffe	—	—	—	—	—	—	431	249·000	1·7
Ox	284	—	—	284	—	280	300	590·000	0·5
Bison	—	—	—	315	—	—	280	816·000	0·3
Roebuck	—	—	—	—	—	168	—	—	—
Zebra	305	305	—	—	—	—	—	—	—
Goat	154	154	—	150	—	151	—	—	—
Ibex	—	—	—	150–180	—	—	—	—	—
Odd-toed Ungulates									
Horse	300	300	—	330–350	—	356	350	680·000	0·5
Ass	300	—	—	360–380	—	357	365	362·000	1·0
Pig	119	119	—	120	—	114	100	81·800	1·2
Hippopotamus	—	—	—	210–250	—	235	235	2715·000	0·09
Rhinoceros	505	—	—	510–550	—	540	576	1810·000	0·3
Elephant	670	—	—	615–628	—	658	600	3625·000	0·16
Zebra	—	—	—	345–375	—	—	—	—	—
Boar	—	—	—	120	—	119	—	—	—
Tapir	—	—	—	400	—	—	—	—	—
Carnivora									
Weasel	—	35	—	—	—	35	35	—	—
Otter	—	—	—	—	—	63	63	8·160	7·7
Polecat	—	63	—	—	—	63	62	1·240	50·0
Puma	—	—	—	92	—	—	—	—	—
Leopard	—	—	—	93	—	—	—	—	—
Marten	—	56	—	—	—	56	90	1·360	66·1
Dog	63	63	—	60	—	63	63	22·500	2·8
Wolf	70	70	—	63	—	92	63	40·700	1·55
Fox	63	63	—	60	—	63	63	6·800	9·3
Bear	210	210	—	240	—	217	120	135·500	0·9
Cat	56	56	—	56	—	50	56	5·430	10·3
Lynx	—	63	—	—	—	63	70	—	—
Panther	—	—	—	—	—	—	63	127·000	0·5
Jaguar	—	—	—	—	—	—	100	113·000	0·9
Lion	98	98	—	105	—	—	110	158·000	0·7
Tiger	—	—	—	105	—	—	110	158·000	0·7
Badger	—	70	—	—	—	63	—	—	—
Ermine	—	—	—	74	—	—	—	—	—
Ferret	42	—	—	—	—	—	—	—	—
Seal	—	—	—	350	—	—	245	—	—
Primates									
Monkey	210	210	—	—	—	—	210	—	—
Baboon	—	—	—	—	—	—	210	22·600	9·3
Gorilla	—	—	—	—	—	—	280	136·000	2·06
Man	280	—	—	280	—	—	280	63·000	4·45
Chimpanzee	—	—	—	260	—	—	—	—	—
Macacus	—	—	—	160–210	—	—	—	—	—
Mandrill	—	—	—	177–210	—	—	—	—	—
Uistiti	—	—	—	84	—	—	—	—	—
Lemur	—	—	—	144	—	—	—	—	—

Table 64. *Gestation time and incubation time.**

Mammals

Species	Gestation time in days	Extreme variation	Investigator
Man	280	—	Hippocrates
,,	272·5	—	Leuckart
,,	272·2	—	Lowenhardt
,,	272·2 (calc. from date of last menstruation)	—	Hasler
,,	280·5 (conception calc.)	—	,,
,,	270	—	Schlichting
,,	279·14 (primipara)	—	Zollner
,,	281·99 (multipara)	—	,,
,,	271·0	—	Ahlfeld
,,	282·5 (for male foetus)	—	Robertson
,,	284·5 (for female foetus both calc. from last menstruation)	—	,,
,,	282·8 (for male foetus)	—	Siegel †
,,	282·0 (for female foetus both calc. from last menstruation)	—	,,
,,	272·6 (for male foetus)	—	,,
,,	267·5 (for female foetus both calc. from one definite coitus)	—	,,
,,	279	—	Hecker
Albino rat	21·6–22·64	—	Stotsenberg
,,	Gestation time may be prolonged from 1 to 6 days if it is simultaneous with suckling	—	King
Norway rat	21·0	—	Lantz
,,	23·5–25·5 (probably suckling)	—	Miller
Guinea-pig	64	—	Draper
Sheep	146	137–162	Schwarz
,,	145–153	—	Sabatini
Cow	275–291 (according to breed)	210–335	Franck-Albrecht and Brody
Horse	334–359	264–420	Ewart
Opossum	13	—	Heuser & Hartmann
Rhinoceros	540	—	Feldman
Elephant	630	—	,,
Jackal (*Anubis pavian*)	210	—	Heinroth
Dog	60	55–68	Schwarz
Pig	120	104–133	,,
Goat	151	—	Asdell
Coyote (*Canis ochropus*)	65	—	Gander
Raccoon (*Procyon californicus*)	65	—	,,
Coatis (*Nasua* ?)	71	—	,,
Opossum (*Didelphys virginiana*)	13	—	,,
Chimpanzee	210–245	—	Dorland & Hubeny

Birds

Species	Incubation time in days	Investigator
Swan	42	Davy
Goose	35	,,
Duck	28	,,
Hen	21	,,

* See also *Tabulae Biologicae*, vol. 6.

† Siegel's figures were obtained during the war on the basis of very accurate data (see also Jolly).

Table 64. *Gestation time and incubation time* (cont.).

Birds (cont.)

Species	Incubation time in days	Investigator
Turkey	28	Davy
Guinea-fowl	30–31	,,
Partridge	27	,,
Pheasant	23	,,
Red grouse	23	,,
Pigeon	14	,,
Turtle-dove	14	,,
Canary	13	,,
Wren	10	,,
Martin (*Hirundo urbica*)	12–13	Meyer
Swift (*Hirundo apus*)	16–17	,,
Eagle-owl (*Bubo maximus*)	21	,,
Goshawk (*Astur palumbarius*)	21	,,
Sparrowhawk (*Accipiter fringillarius*)	21	,,
Stockdove (*Columba oenas*)	17	,,
Turtledove (*Columba turtur*)	16–17	,,
Pheasant (*Phasianus colchicus*)	24–26	,,
Cock-of-the-wood (*Tetrao urogallus*)	28	,,
Black grouse (*Tetrao tetrix*)	21	,,
Pheasant (*Tetrao perdix*)	21	,,
Swan (*Cygnus olor*)	35–42	,,
Wild duck (*Anas boschas*)	29	,,
Humming-bird	12	Milne-Edwards
Hen	21	,,
Duck	25	,,
Cormorant	25	,,
Guinea-fowl	25	,,
Turkey	27	,,
Goose	29	,,
Peacock	31	,,
Swan	42	,,
Cassowary	65	,,
Turkey	29	Evans
Guinea-fowl	25–26	,,
Duck	28	,,
Partridge	25	,,
Humming-bird	12	Schenk
Calcutta hen	27	,,
Peacock	31	,,
Stork	42	,,
Cassowary	65	,,
Goose	29	,,
Duck	21	,,
Guinea-fowl	21	,,
Blackbird (*Turdus merula*)	15	Evans*
Redstart (*Ruticilla phoenicurus*)	14	,,
Robin (*Erithracus rubecula*)	13·5	,,
Sedge-warbler (*Acrocephalus phragmitis*)	15	,,
Great tit (*Parus major*)	14·9	,,
Pied wagtail (*Motacilla lugubris*)	14·5	,,
Swallow (*Hirundo rustica*)	15·5	,,
Skylark (*Alauda arvensis*)	13·5	,,
Common tern (*Sterna fluviatilis*)	21·5	,,
Redshank (*Totanus calidris*)	23	,,
Stormy petrel (*Procellaria pelagica*)	36	,,

* According to Evans the data in Giglioli's report are not reliable.

Table 64. *Gestation time and incubation time* (cont.).

Species	Incubation time	Investigator
	Birds (cont.)	
Condor (*Sarcorhamphus gryphus*)	54 days	Broderip
Buzzard (*Buteo vulgaris*)	31 ,,	Anonymous
Turkey	26–29 ,,	Vignes
Hen	19–24 ,,	,,
Duck	29–32 ,,	,,
Pigeon	17–20 ,,	,,
Rhea (*Rhea americana*)	29 ,,	Rozanov
Buzzard (*Buteo buteo*)	30 ,,	Groebbels & Möbert
Kestrel (*Cerchneis tinnunculus*)	28·5 ,,	,, ,,
Pheasant (*Phasianus colchicus*)	25 ,,	,, ,,
Nightingale (*Turdus philomelos*)	13·5 ,,	,, ,,
Yellow-hammer (*Emberiza citrinella sylvestris*)	12·5 ,,	,, ,,
Chaffinch (*Fringilla coelebs*)	13 ,,	,, ,,
Goldfinch (*Acanthis cannabina*)	11 ,,	,, ,,
Robin (*Erithracus rubecula*)	14 ,,	,, ,,
Hedge-sparrow (*Prunella modularis*)	12 ,,	,, ,,
Lesser whitethroat (*Sylvia curruca*)	11·5 ,,	,, ,,
Sedge-warbler (*Acrocephalus phragmitis*)	12·5 ,,	,, ,,
Golden vulture (*Gypaetus barbatus*)	53 ,,	Schumann
Common pigeon: 1st egg of the clutch	16·42 ,,	Cole & Kirkpatrick
,, ,, 2nd egg of the clutch	16·89 ,,	,, ,,
	Reptiles	
Marine turtle (*Thalassochelys corticata*)	47 days	Tomita
Python (*Python molurus*)	2½ months	Cunningham
Loggerhead turtle (*Caretta caretta*)	64 days	Hildebrand & Hatsel
Green turtle	8½ weeks	Bergtold
Loggerhead turtle (*Caretta caretta*)	8½ ,,	,,
European tortoise (*Emys europaea*)	20–44 ,,	,,
Tuatara (*Sphenodon*)	52 ,,	Dendy
Crocodile	12 ,,	Bergtold
Alligator	8½ ,,	,,
Python (*Python reticulatus*)	6–8 ,,	Detmers
Python (*Python molurus*)	10 ,,	Bergtold
Black snake	8½ ,,	,,
Fox snake	7½–8½ ,,	,,
Corn snake	6–8 ,,	,,
Yellow rat snake	11 ,,	,,
Ring snake	6 ,,	,,
Milk snake	8 ,,	,,
King snake	6–8 ,,	,,
Coral snake	8½ ,,	,,
	Fishes	
Dogfish (*Scyllium catulus*)	157–178 days (av. 169)	Bolau
,, (*Scyllium canicula*)	234–280 ,, (av. 261)	,,
Ray (*Raia batis*)	9–10 months	Beard
Herring	12 ,,	Hoffmann
Sturgeon (*Acipenser stellatus*)	44–80 hours	Derjavin
Plaice	6–15 months	Dannevig
Flounder	3–7 ,,	,,
Trout (*Salmo fario*)	205 days (2°)	Haempel
,, ,,	82 ,, (5°)	,,
,, ,,	41 ,, (10°)	,,
American flounder (*Pseudopleuronectes americanus*)	26 ,,	Scott
Japanese salmonoid (*Plecoglossus altivelis*)	10–25 days	Nakai
Pike	9 ,,	Kvasnikov

Table 64. *Gestation time and incubation time* (cont.).

Species	Incubation time	Investigator
Crustacea		
Water-flea (*Daphnia pulex*)	75–85 hours	Ramult
Lobster	10 months	Allen
Amphipod (*Gammarus chevreuxi*)	8–9 days	Ford & Huxley
Insects		
Cockroach (*Periplaneta orientalis*)	75 days	Zabinski
Tent-caterpillar moth (*Malacosoma americana*)	9·5 months	Rudolfs
Sarcophagidae	Less than a day	Imms
House fly (*Musca domestica*)	8–12 hours	,,
Lepidoptera	Several months	,,
Phasmidae	2 years	,,
Ant (*Aphaenogaster fulva*)	17–22 days	Fielde
,, (*Myrmica rubra*)	23–24 ,,	,,
Dragonflies	3 weeks	Needham
Oriental peach-moth (*Laspeyresia molesta*)	4 days	Snapp & Swingle
Butterfly (*Diacrisia virginica*)	5·5 ,,	Johannsen
Swine-louse (*Haematopinus sui*)	14 ,,	Weber
Beet Leafhopper (*Eutettix tenellus*) ...	10–50 days	Severin
Stonefly (*Perlodes mortoni*)	91 days	Percival & Whitehead
,, (*Perla carlukiana*)	59 ,,	,, ,,
,, (*Nephelopteryx nebulosa*)	20 ,,	,, ,,
Mayfly (*Siphlurus armatus*)	15 weeks	,, ,,
,, (*Ecdyurus venosus*)	15 days	,, ,,
Molluscs		
West African land-snail (*Achatina variegata*)	40–60 days	Pycraft
Periwinkle (*Littorina littorea*)	6 days	Hayes
Worms		
Roundworm (*Ankylostoma duodenale*) ...	1–3 days	Looss

Table 65. *Time-relations of early development.*

AMPHIBIANS

Wilson's figures (15° C.):

Species	Fertilisation to first cleavage (hrs.)	Cleavage periods (min.)			Gastrulation (hrs.)		Appearance of external gills (days)	Adult (days)
		1st	2nd	3rd	Beginning	End		
Salamander (*Amblystoma punctata*)	10	110	100	100	60	78	19	100
Frog (*Rana temporaria*)	3·5	75	60	50	42	50	14	70
Small wood-frog (*Chorophilus triserratus*)	1·5	30	40	30	11	13	3·5	30

Hertwig's figures (14° C.):

Rana temporaria	Days after fertilisation
Gastrulation	1·3
Formation of medullary plate ...	4·2
Closure of medullary folds ...	5·5
Appearance of tail-bud	6·8
Appearance of tail and gills ...	8·6
Appearance of tail-fin	11·0
Beginning of operculum	13·4

Table 65. *Time-relations of early development* (cont.).

MOLLUSCS

A. Richards' figures:

(Opisthobranch)

	Planorbis		*Haminea virescens*	
	Time from fertilisation	Intervals in minutes	Time from fertilisation	Intervals in minutes
Fertilisation	0		0	
		90		85
1st cleavage	90		85	
		75		51
2nd cleavage	165		136	
		75		59
3rd cleavage	240		195	
		75		50
4th cleavage	315		245	
		60		40
5th cleavage	375		285	

ASCIDIAN

E. G. Conklin's figures:

	Cynthia	
	Time from fertilisation	Intervals in minutes
Fertilisation	0	
		40
1st cleavage	40	
		30
2nd cleavage	70	
		20
3rd cleavage	90	
		20
4th cleavage	110	
		20
5th cleavage	130	
		20*
6th cleavage	150	
		20
7th cleavage	170	
		20
8th cleavage (218 cells)	190	
		120
Hatched tadpole	310	

* Beginning of gastrulation.

The inner significance of the length of embryonic life relative to the life-span is most obscure. Some interesting remarks have been made by Moulton, who has pointed out that over the whole life-span, the chemical changes are much more intense in the earliest periods, i.e. pre-natal and to a certain extent post-natal. This reminds

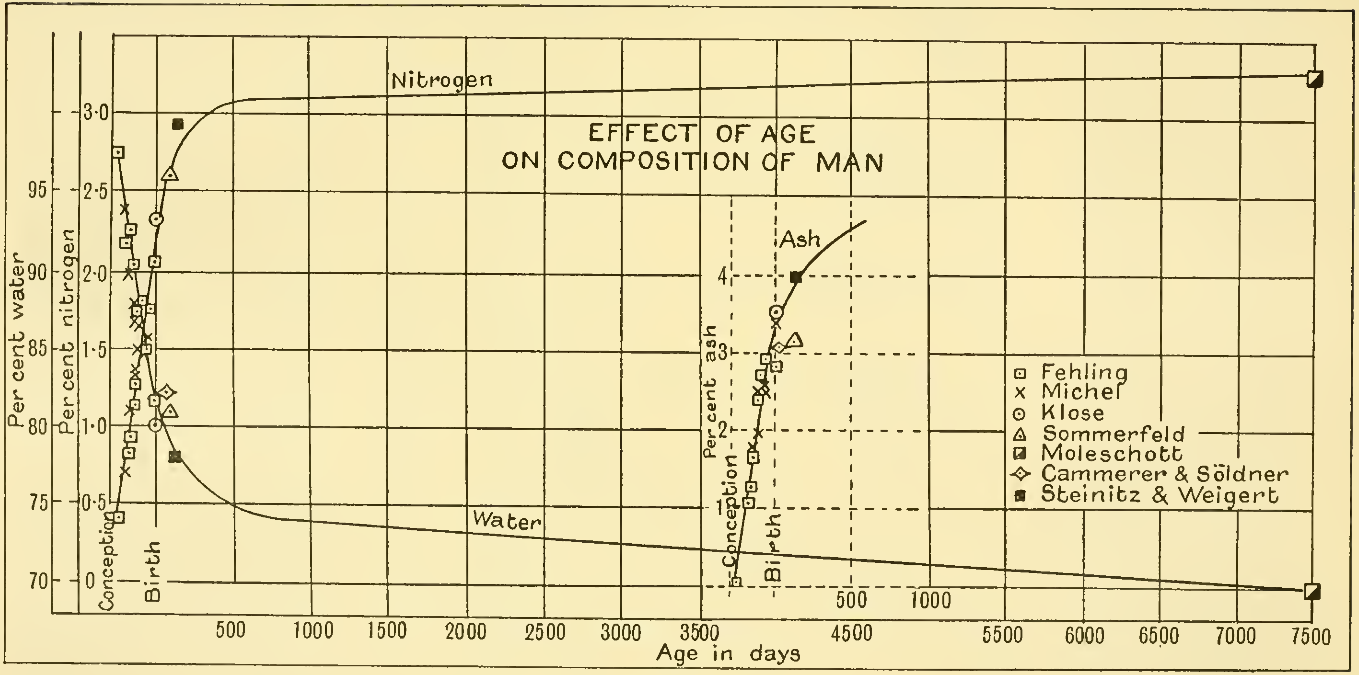
EFFECT OF AGE
ON COMPOSITION OF MAN
Nitrogen
Water
Ash
Per cent water
Per cent nitrogen
Per cent ash
95
90
85
80
75
70
3·0
2·5
2·0
1·5
1·0
0·5
0
4
3
2
1
Conception
Birth
Conception
Birth
500
1000
1500
2000
2500
3000
3500
4500
5500
6000
6500
7000
7500
500
1000
Age in days
▫ Fehling
× Michel
⊙ Klose
△ Sommerfeld
▨ Moleschott
◇ Cammerer & Söldner
■ Steinitz & Weigert

Fig. 73.

us that Murray's law only applies to the embryonic period, and must not be extended beyond it (see p. 548). A typical graph is that shown in Fig. 73 where the composition of the whole human being as regards water, nitrogen and ash, is considered. The point of sudden cessation of intense chemical redistribution was termed quite logically by Moulton "chemical maturity", and this point, he found, bore a fairly constant relation to the total life-span, as appears from the following table:

Table 66.

Moulton's figures:

Species	Average length of gestation period (days)	Average length of life (years)	Average conception age at maximum of 3rd growth cycle (Brody & Ragsdale) (days)	Conceptional age at chemical maturity (days)	Part of life-span passed at chemical maturity % of total life-span	Constitution of body at birth		
						Water %	Protein %	Ash %
Man	285	80	5300	1285	4·4	82	14	3
Cow	285	25	850	435	4·6	76	18	4
Pig	120	20	200	345	4·6	82	13	3
Guinea-pig	64	7	145	114	4·6	78	17	4
Dog	61	17	—	261	4·3	82	14	3
Cat	60	11	—	160	3·9	83	13	3
Rabbit	31	10	185	—	—	84	13	2
Rat	25	4	86	75	4·5	88	10	2
Mouse	20	4	62	—	—	86	11	3

This, however, was the only relation which did show any constancy, and very little can be deduced about an animal if only its gestation time, or conversely, only its average length of life, is known. Even its composition at birth is not related simply to any of the other variables. These facts, to which Moulton was the first to draw attention, illustrate the truth of the statement just made, namely, that the act of birth or hatching is a comparatively unimportant one in the life of the individual. Probably the time at which it takes place in the life-span has been much involved with the adaptations due to different modes of life, while yet the underlying process of physico-chemical maturation has remained unaffected.

Extremely few researches have been done on these problems. In 1926 I estimated the non-protein nitrogen in a variety of bird embryos, of different incubation periods, with a view to ascertaining whether the rhythm of chemical differentiation went on at a constant rate in all cases. The curve shown in Fig. 74 is from the data on non-protein-nitrogen of White Leghorn embryos. Similar data were obtained for two other races of domestic hen (21 days), the pigeon (18 days), the guinea-fowl (25 days), the duck (26 days), the partridge (27 days), and the turkey (28 days). The results were related to weights of embryos equal to those of the chick embryo on different days, and as can be seen, the values for the other embryos fell uniformly on the chick curve. The length of incubation would thus appear to be a tune played as it were, "adagio" in the turkey and "allegro" in the pigeon.*

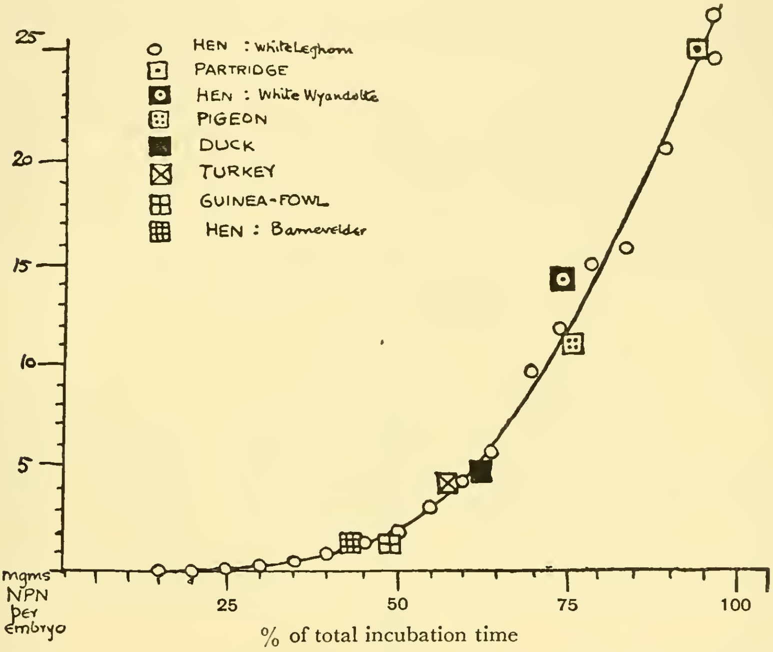

Fig. 74.

The possible adaptive significance of incubation time has been shown remarkably in a paper by Friedmann, who found that the incubation times of the cowbirds, such as *Agelaioides*, *Molothrus* and *Tangavius*, varied according to their degree of parasitism. *M. ater*, which is very parasitic, has an incubation time of 10 days (the shortest known), *M. bonariensis,* less parasitic, takes 11·5, and *M. rufo-axillaris,* still less so, takes 12·5 to 13 days.

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* For this it was necessary to assume that the pre-natal growth-curves for wet weight were alike, an assumption which was subsequently shown to be legitimate by Kaufmann. The instantaneous percentage growth-rates of the pigeon and the hen do not begin to differ until after hatching; and this holds, according to her, for heart, liver and eye, as well as for the whole body. The cells of the pigeon embryo, however, are smaller than those of the chick by about 30%.

No consideration of the questions involved in incubation and gestation time would be complete without mention of the work of Rubner. Rubner found that, if a graph is constructed having the durations of pregnancy of different groups of animals as abscissae and the respective birth-weights of their young as ordinates, the resulting curve is quite smooth and regular. These facts, which have already been discussed, are shown in Fig. 75, taken from Rubner. A glance shows that the only exception among the animals which Rubner chose is man, who develops very slowly, and does not attain at birth more than a quarter of the weight he should if he resembled other animals. Rubner considered the anthropoid apes to be more like animals than man in this matter, but an observation of Heinroth's makes this doubtful. It has often been pointed out that small birth-weight is probably an adaptive feature of considerable advantage to the human female. The actual figures are as follows:

Table 67.

Animal	Period in days during which the new-born doubles its weight		Gestation period in days (Thiel; Landois; Klimmer)	Weight of maternal organism in kilos	Weight of new-born in kilos	Weight of new-born in % of maternal weight
	(Bunge)	(Abderhalden)				
Horse	60	60	340	450	50·0	11·0
Cow	47	47	285	450	35·0	8·5
Sheep	12	15	154	50	3·9	7·8
Man	180	180	280	55	3·0	5·5
Pig	16	14	120	80	2·4	3·0
Dog	8	9	63	—	—	2·0
Cat	9	9	56	—	—	—
Rabbit	6	6	28	—	—	—
Guinea-pig	—	—	67	0·62	0·087	14·2
Mouse	—	—	21	0·02	0·0017	8·5
					Average	7·6

Rubner studied the heat production of the new-born animals, and found that the larger the birth-weight the smaller the number of Calories put out by 1 kilo of body-weight per day. Thus for a new-born animal weighing 50 kilos, 1 kilo of body-weight would produce 34·2 Calories, for one weighing 25 kilos the production would be 42·6 Calories, for one of 12·5 kilos it was 60 Calories, and for one of 6·25 kilos it was 66·6 Calories. The explanation of this obviously was that, since no supply of energy can be built up *in toto* into potential energy stored in the tissues, but must to some extent be wasted in upkeep metabolism, a wastage would be expected in the case now under consideration, and as the relative surface of the new-born is larger the smaller it is itself, this wastage would be expected to be greater. Rubner then assumed that the heat production of the embryo was about seven-tenths of the new-born animal, presumably on the basis of premature birth figures, but also on theoretical grounds drawn from the embryo's existence in a thermostatic hydrosphere unaccompanied by much muscular or digestive exertion. This assumption seems a dangerous simplification, in view of the peak in basal metabolism which we now know to exist in some animals before and in some animals after birth. However, Rubner calculated the intra-uterine heat production as follows:

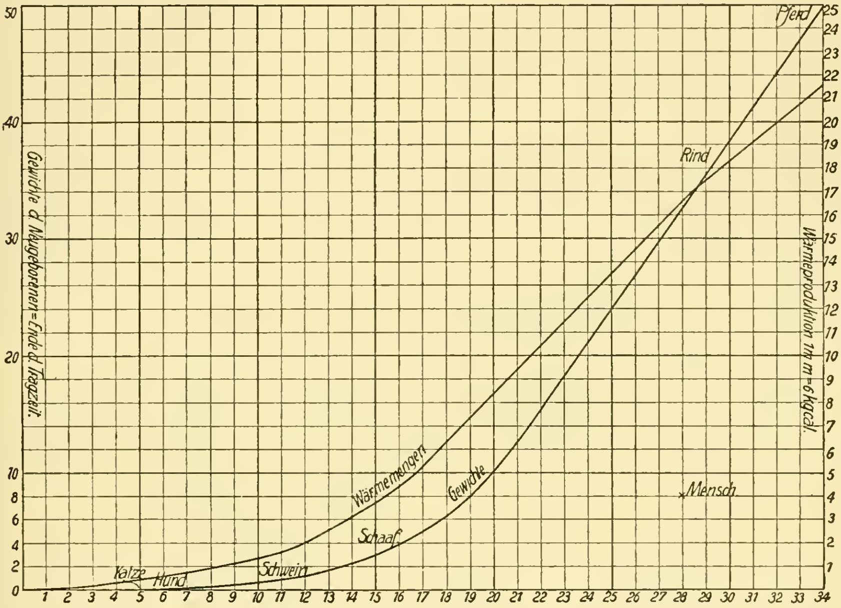

Fig. 75.

Birth-weight of animal (kilos)	Cals. put out by 1 kilo* per day
50·0	23·9
25·0	29·8
12·5	42·0
6·25	46·6

* I.e. seven-tenths of the post-natal figures.

Averaging the whole of development, Rubner divided each of these figures by 2, and, taking the duration of appreciable development at six-tenths of the whole (the first four-tenths being a negligible quantity), he multiplied each by six-tenths of the total gestation time in days, thus:

Birth-weight of animal in kilos	Total gestation time		Average no. of Calories put out by 1 kilo of finished embryo throughout development
		×6/10	
50·0	340	204	2631
25·0	250	150	2235
12·5	205	123	2583
6·25	177	106	2470
		Average ...	2480

Thus 1 kilo of an imaginary animal (it would be very like a horse), which weighed 50 kilos at birth, would eliminate 2631 Calories during its whole gestation period, while 1 kilo of an imaginary animal which weighed 6·25 kilos at birth would eliminate 2470 Calories, its relatively more intense heat output being compensated for by its shorter gestation period. Rubner called this relation the "fundamental law of intra-uterine developmental energy".

In the above discussion, we were dealing with imaginary examples, but Rubner calculated out the figures for actual animals, as follows:

Animal	Birth-weight	Average no. of Calories put out by 1 kilo of embryo throughout development
Horse	50·0	2028
Cow	35·0	1915
Sheep	3·9	2728
Pig	2·4	2210
Dog	—	2318
	Average ...	2240

Now if we assess the calorific value of the formed kilogram of finished embryo at 1504 Calories, 2480 plus 1504 Calories, i.e. 3984 Calories, are required for the formation of 1 kilo of embryo during intra-uterine life in the higher mammals. Rubner spoke of a "growth-quotient" in this connection, defined as follows:

$$\frac{\text{Energy stored in the tissues} \times 100}{\text{Energy stored plus energy given off as heat}}.$$

In the case of pre-natal life, it was 1500/4000, i.e. about 38 per cent. There is a certain contradiction here between this efficiency datum and the conclusions of other workers (for which see the section on energy relations), and it is quite certain that the efficiency changes during development. Rubner's law of constant energy requirement during gestation time does not equate well with the findings of Brody, Moulton and others, but it must be remembered that the "law" rests on a very inadequate basis, nothing more, in effect, than a few rough calculations in which some doubtful assumptions are involved.

Rubner emphasised the fact that the value of about 4000 Calories, which seem to be required to make one kilo of tissue during embryonic development, is lower than the corresponding value of about 4800 for post-natal life. From this it would appear that the efficiency is greater before birth than afterwards, but this statement needs much qualification. Rubner also showed that the energy consumed per kilo in doubling the birth-weight is approximately the same for different animals. Thus:

Animal	Energy consumption per kilo in doubling the birth-weight, Cals.
Horse	4,512
Cow	4,243
Sheep	3,926
Pig	3,754
Cat	4,304
Rabbit	4,554
Average ...	4,215
Man	28,864

Robertson has shown that the generalised form of this rule would be

$$\frac{E}{x} = a.\log x + b,$$

where E is the energy-consumption, x the weight of the animal and a and b constants which are the same for all species except man.

But we have digressed already too far from the problem of growth. Before taking up the effects of external agents on the growth-rate, it will be worth glancing for a moment at the relations between gestation time and the life-span. Buffon in the eighteenth century and Flourens in the nineteenth maintained that the life-span was five or six times the youth-period (see Lusk), but Weissmann showed that there were too many exceptions to this rule to make it of any use. Rubner's book contains a discussion of this matter, from which it is to be concluded that, at present, there is no rule which covers all the cases. [See also Hollis and Szabó.]

Rubner's "second law", in which he laid down that the total amount of heat eliminated from birth to death in all the higher mammals is the same, i.e. about 191,600 Calories per kilo, with the exception of man, for which the value was 725,700 Calories per kilo, has often been criticised adversely, and does not here concern us. But it is to be observed that it contradicts the lack of relation found by Moulton and others between simple gestation time and life-span, in that, according to Rubner, a constant percentage of the total heat eliminated during life has been eliminated at birth, i.e. 2500 out of 191,600 or 1·305 per cent. This proportion does not, of course, hold in the case of man, but will be greater because of his prolonged stay *in utero*.

Our immediate aim must now be to examine the effects of various physical influences upon the rate of growth of the living embryo, for by the aid of such a study one may hope to penetrate further into the fundamental nature of the process. That chemical influences can also exert a great effect on the growth of the embryo is obvious, but they will be dealt with in succeeding chapters, such as the sections on vitamines, and general metabolism. At present the discussion will be strictly confined to the effects of radiant energy (heat and light) on the rate of growth, and all teratological considerations will be kept in the background.

2·14. The Effect of Heat on Embryonic Growth

The accelerating influence of rise of temperature on embryonic growth was known to William Harvey, though in an unformulated kind of way, for referring to differences in gestation time, or what he calls "the diversity of going with Child", he says: "For the same thing befalls them as happeneth to Plants, whose fruits and seeds, do more slowly and seldom arrive to maturity in cold Countries, than do other Plants of the same kind which are in a fat and warme soile. So Orenges in England adhere to the trees almost two whole years together, before they come to maturity: and Figgs also scarce ever arrive at any perfection here, which are ripe in Italy twice or thrice a year. And the like befalleth the fruits of the Womb". But perhaps the earliest quantitative observation of the effect of heat on development was that of Gaspard, who in 1822 constructed the following table:

Temperature (°C.)	Developmental time of snail's eggs in days
6–8	45
12	38
20	21

Gaspard also worked on frog's eggs. Davy and Coste, both in 1856, published some figures showing the acceleration of development of the eggs of the salmon in warmed water. Most of the early work was, of course, fragmentary, and for various reasons unsatisfactory. Thus Philipeaux in 1871 observed that the hatching time of axolotl eggs was shortened from 25 to 8 days as warmth increased but he did not take the temperatures, and Vernon stated in 1895 that the optimum temperature for the development of the embryos of echinoidea was from 7° to 22°, outside which limits they rapidly became abnormal. Semper found that nauplii of *Branchus* and *Apus* hatch out at a temperature of 30° in less than 24 hours, whereas at 16–20° they require some weeks. Again, lobster larvae reared at 23–27° passed the fourth moult in about 10 days, i.e. 3 days earlier than larvae reared at 19° C., according to Herrick. Cuénot gave the following figures for the hatching time of the locust:

°C.	25	20	15	10
Days ...	50	55	60	65

Table 68, partly from Davenport, shows the time taken in embryonic development at various temperatures for certain fishes. As we shall see further in the section on resistance and susceptibility, there are to be found among embryos very obvious adaptations to the environment in which they are to grow; thus Rauber has shown that the eggs of minnows and salmon which develop during the winter will not grow at all at temperatures much above 12–15°, but will do so at 0°. The critical points below and above which normal development will not go on have been determined for amphibians and birds by various observers, and are tabulated in Table 68. The optimum temperature may be regarded as that at which the smallest number of abnormal embryos are produced; on each side of it the amount of teratological effect will more or less rapidly increase, while at the same time the rapidity of development will on the one hand be increased and on the other hand be retarded. When a certain

Table 68.

Days taken in development.

Temperature of water (°C.)	Cod (Earll)	Herring (Meyer and Kupffer)	Shad (Rice)
−2– 0·0	30·0	—	—
0– 1·9	32·5	—	—
2– 3·9	22·0	40	—
4– 5·9	16·0	—	—
6– 7·9	13·0	15	—
8– 9·9	—	—	—
10–11·9	—	11	—
13·5	—	—	11
20–23·0	—	—	3·5
14–20·0	—	7	—

Early quantitative observations.

Time taken to reach definite stage, 38° taken as unity (*Rana temporaria*)

Temperature °C.	20	21	22	23	24	25	26	27	28	29	30	31
Edwards' figures	0·01	0·01	0·02	0·02	0·03	0·03	0·04	0·07	0·12	0·15	0·35	0·55

Temperature °C.	34	35	36	37	38	39	40	41	42
Féré's figures	0·65	0·80	0·72	—	1·0	1·06	1·25	1·51	0·0

Lillie & Knowlton's figures:

Increase in length in mm. from 24 to 48 hours after hatching.

°C.	*Rana virescens*	*Bufo lentiginosus*
9–10·9	4·5	3·0
11–12·9	5·3	5·3
13–14·9	4·3	15·5
15–16·9	—	16·3
17–18·9	9·5	—
19–20·9	19·8	21·2
21–22·9	—	—
23–24·9	—	41·3
25–26·9	31·5	39·0
27–28·9	40·0	—
29–30·9	47·5	56·8
31–32·9	40·2	55·3
33–34·9	43·5	—

Higginbottom's figures. Frog (*Rana fusca*):

	Temperature °F.			
Date	60	56	53	51
March 11	Egg	Egg	Egg	Egg
20	Hatch	—	—	—
23	External gills	—	—	—
25	—	Hatch	—	—
27	Internal gills	—	—	—
28	—	External gills	—	—
31	—	—	Hatch	Hatch
April 4	—	—	External gills	—
6	—	Internal gills	—	External gills
10	Big tadpole	—	—	—
May 22	Metamorphosis	Big tadpole	Internal gills	Internal gills
Aug. 18	—	Metamorphosis	—	—
28	—	—	Metamorphosis	—
Oct. 31	—	—	—	Metamorphosis

Table 68 (*cont.*).

Marcus' figures. Sea-urchin (*Strongylocentrotus lividus*).

Days from fertilisation	Temperature ° C.		
	9	17–19	22
0	Egg	Egg	Egg
7	Blastula	Blastula	Gastrula
22	Blastula	Mesenchyme	Gastrula
29·5	Blastula	Gastrula	Pluteus
47·5	Mesenchyme	Pluteus	—
55·5	Beginning of gastrulation	Old pluteus	—
70·5	Gastrulation	—	—
80	Gastrulation	—	—
95·5	End of gastrulation	—	—
118·5	Prismatic gastrulation	—	—

Environmental temperatures.

Species	Temperature ° C.			Investigator
	Minimum	Optimum	Maximum	
Minnow	0	—	12–15	Rauber
Salmon	0	—	12–15	,,
Sea-urchin (*Echinus esculentus*)	—	17–22	—	Vernon
Frog (*Rana palustris*) ...	2·5	—	—	Morgan
,, (*Rana virescens*) ...	3	30	—	,,
Toad (*Bufo lentiginosus*) ...	6	32	—	Lillie & Knowlton
Hen (*Gallus domesticus*) ...	25	38	42	Rauber
Salamander (*Amblystoma tigrinus*)	0	—	—	Lillie & Knowlton
Frog (*Rana fusca*)	0	—	—	Schultze
Hen (*Gallus domesticus*) ...	28	35–39	43	Kaestner
,, ,, ...	29	—	—	Prévost & Dumas
,, ,, ...	20·5	—	—	Edwards
,, ,, ...	—	37·6 (1st week) 38·3 (2nd ,,) 39·1 (3rd ,,)	—	Eccleshymer
Green bug (*Toxoptera graminium*)	1·65	—	—	Hunter & Glenn
Texan cattle-fever tick (*Margaropus annulatus*)	7·5	—	—	Hunter & Hooker
Brown-tailed moth (*Euproctis anysonhea*)	11	—	32	Sanderson
Bombycid moth (*Samia cecropia*)	11	—	—	,,
Mealworm (*Tenebrio molitor*)	9	—	—	,,
Colorado potato-beetle (*Leptinotarsa decemlineata*)	12	—	32·5	,,
Pine bug (*Dendrolimus pini*)	14	—	28	Regener
Hen (*Gallus domesticus*) ...	—	38·3*	—	Phillips & Brooks
Rhea (*Rhea americana*) ...	—	38·5	—	Rozanov
Locust (*Melanoplus differentialis*)	18	36	—	Bodine
Locust (*Melanoplus femur-rubrum*)	18	36	—	,,

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* Atkinson considers that better results in artificial incubation are obtained when the heat is conveyed to the eggs by conduction, not radiation, e.g. by covering them with a thin sheet of rubber.

Table 68 (*cont.*).

Environmental temperatures (cont.).

Species	Temperature ° C.			Investigator
	Minimum	Optimum	Maximum	
Japanese cuttlefish (*Ommastrephes sloani*)	12	15	18	Sasaki
Frog (*Rana fusca*)	—	20 rising to 24	—	Hertwig*
Toad (*Bufo lentiginosus*) ...	—	28 ,, 31	—	,,
Frog (*Rana fusca*)	—	—	23 rising to 30	King*
,, (*Rana esculenta*) ...	—	—	33 ,, 34	,,
Toad (*Bufo lentiginosus*) ...	—	—	33 ,, 38	,,
Roundworms:				
(*Toxascaris limbata*) ...	6–7	—	38–40	Zavadovski & Sidorov
(*Ascaris megalocephala*)...	6–7	—	38–40	,, ,,
(*Ascaris suilla*)	7–8	—	36–38	,, ,,
(*Ankylostoma duodenale*)	10	20–30	32	Looss
,, ,, ...	—	21–25	—	Svensson
Trematode of Japanese Bilharziosis (*Schistosomum japonicum*)	—	27·5	—	Miyakawa
Japanese teleost (*Hypomesus olidus pallas*)	6·0	—	17·5	Nakai
Goosefish (*Lophius americanus*)	4·0	10·0	18·0	Berrill
Plaice (*Pseudopleuronectes americanus*)	—	4·5	—	Scott
Salmon (*Salmo irideus*) ...	3·0	6·0	12·0	Kawajiri

* Hertwig's and King's figures are interesting because *Rana fusca* and *temporaria* lay early in spring when the water is often freezing: *Rana palustris* and *esculenta* later, and *Bufo lentiginosus* later still. Giglio-Tos has emphasised the importance of this in ecology.

degree of external heat is reached, the mechanism of development will be irreversibly interfered with and the growth-rate will suddenly drop. On the cold side the growth-rate will fall off steadily. It is necessary to distinguish these two types of effect, for it is only the latter that concerns us here. According to the kinetic theory, rise in temperature leads inevitably to an increased vigour of molecular movement, and, as is well known, the extent to which this happens gives a measure of the extent to which the process under examination is physical or chemical. For in the former case the amount of increase in molecular motion will only exert a direct effect in speeding up the process, but, if the possibility of a chemical combination is present to complicate matters, the effect will be much greater. The older workers believed on these grounds that it would be very simple to determine whether the nature of any given "master reaction" in living matter was physical or chemical, but maturer consideration and extended experiments have shown that such determination is attended with very great difficulty. Bayliss has drawn attention forcibly to this. The position as regards the embryonic growth-process is therefore doubtful, and, although its temperature coefficient has been many times estimated, we cannot yet be certain what the real significance of this is. Nevertheless, the recent researches of Crozier and his school have brought us nearer to a sound judgment upon the matter.

2·15. Temperature Coefficients

The older quantitative observations were few in number and not very accurate; we owe them to Féré; Lillie & Knowlton; Higginbottom; Driesch; Chambers; Edwards; Semper; and Bury. Some of them are shown in Table 68. They were too few in number to lead to any well-based conclusions, though they certainly demonstrated the fact that, within certain limits, the higher the temperature, the higher the growth-rate. But the classical paper on this subject is that of O. Hertwig, who in 1898 subjected developing frog embryos to various temperatures. He had been preceded by Baudrimont & de St Ange, who, as early as 1846, had observed the accelerating effect of temperature on the developing egg of the frog. His figure, which has often been reproduced, is shown as Fig. 76, and from it one can easily see that the time taken to reach the seventh stage, for instance, is 16·7 days at 20° but 55·6 days at 10°. The time taken at the lower temperature, therefore, is just 3·33 times as long as that at the higher temperature, so x^{10} of the van't Hoff equation

$$\frac{V(t+n)}{Vt} = x^n$$

will be 3·33, where Vt is the reciprocal of the weight gained at a certain temperature and $V(t+n)$ is the reciprocal of the weight gained at n degrees higher temperature. Therefore $10 \log x = \log 3{\cdot}33 = 0{\cdot}5224$; therefore $\log x = 0{\cdot}05224$ and $x = 1{\cdot}128$. In other words, if m days are taken to complete a certain stage of development at 10°, it will take $m \times 1{\cdot}128^n$ days when the temperature is n degrees less for the same stage to be arrived at. D'Arcy Thompson calculated all the values of Hertwig's experiments from this simple exponential formula, and obtained a series of curves convex to the abscissa, which showed fair agreement with those plotted from the experimental observations.

Here 1·128 is the temperature coefficient for 1° (in the common terminology Q_1) but, as it is usual to employ the expression Q_{10} as

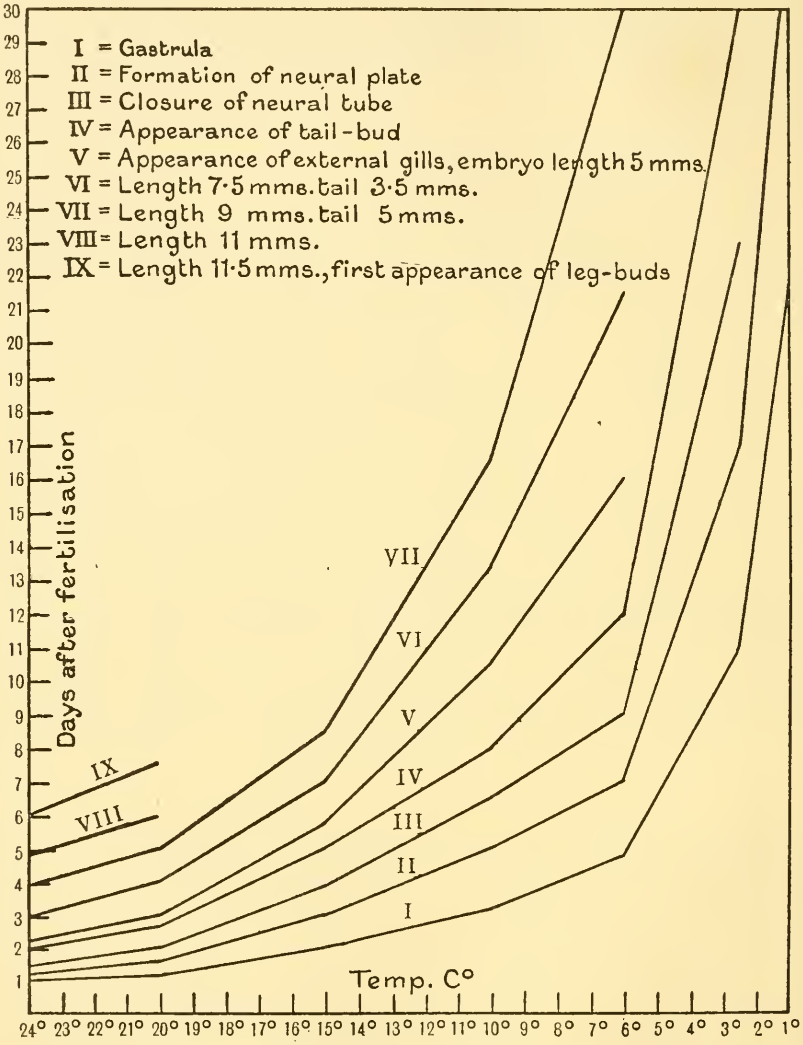

Fig. 76.

the temperature coefficient, the value for the frog is $1·128^{10}$, i.e. 3·34, or, in other words, the time taken to reach a definite stage at 20° multiplied by 3·34 will give the time taken to reach the same stage at 10°. Thus the velocity of the process is more than tripled by a rise of 10° in the temperature, as would ordinarily be expected in the case of a chemical reaction. Judging from Hertwig's curves alone, one should theoretically be able to increase the speed of the growth to infinity by heating the system up, but this of course is not the case, and Fig. 77 taken from Fauré-Fremiet's work on *Sabellaria* eggs shows how, after a certain temperature is reached, the growth-rate may get slower again, owing to the destructive effects of excessive heat on protoplasm. The subject was thoroughly gone into, apparently at Abegg's suggestion, by Peter in 1905, who made many measurements of the development of echinoderm eggs and calculated out the temperature coefficients of Hertwig's results more correctly. His results were as follows:

Table 69.

	Q_{10}			
	Sphaerechinus	*Echinus*	*Rana fusca*	Q_1
2-cell stage	2·137	—	First cell-division	2·040
4-cell stage	2·352	2·277	Second cell-division	2·404
8-cell stage	2·402	1·758	Third cell-division	2·264
16-cell stage	2·437	1·956	Gastrula	2·490
32-cell stage	2·277	2·509	Medullary plate	3·487
Blastula	2·177	2·272	Closure of medullary folds	3·701
First mesoderm	2·625	2·042	Appearance of tail-bud	4·102
First spicule	1·575	1·830	Appearance of external gill	3·151
Full spicules	1·600	1·609	Appearance of tail-fin	3·138
Prisma	—	1·831	Beginning of operculum	3·245
Young pluteus	—	1·707		
Old pluteus	—	1·546		

From these figures Peter concluded that the average temperature coefficients of the three embryos were not very different, i.e. *Sphaerechinus* 2·15, *Echinus* 2·13, and *Rana* 2·86. He regarded the similarity between the values for the echinoderms as significant. He observed also that Q_{10} was not quite a constant: thus between 2·5 and 14·5° it was 3·28 (average) and between 14 and 24° it was 2·26; or in other words that the development at low temperatures was rather slower than it ought to have been if the same temperature coefficient held for all temperatures. Next Peter noted that Q_{10} was not the same for each stage of development, for, on the whole, the average coefficient for the cleavage processes was higher than that for the later events. This rule, however, only held for the two echinoderms studied and not for the frog, thus:

	Q_{10}	
	Cleavage stages	Later stages
Sphaerechinus	2·29	2·03
Echinus	2·30	2·08
Rana	2·23	3·34

Such a comparison obviously suggested interesting conclusions in view of the fact that the two alecithic eggs behaved in the same way

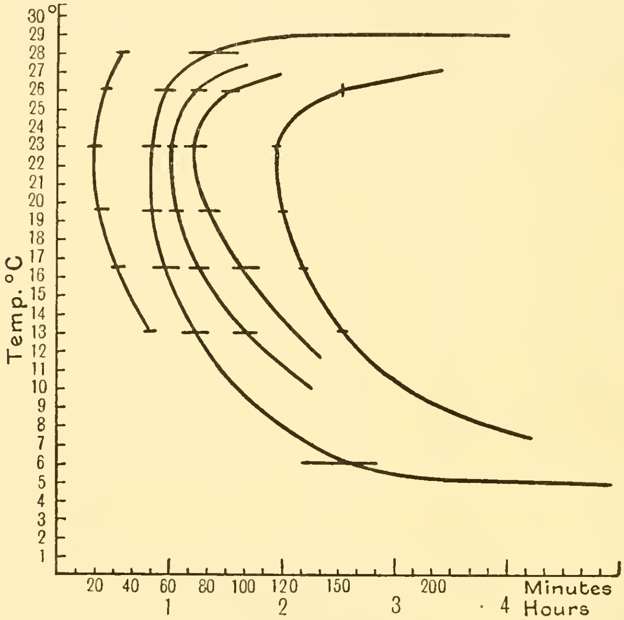

Fig. 77.

while the yolk-laden one did not. Peter's final procedure was to set side by side a quotation from van't Hoff, in which chemical reactions were said to be increased $2\frac{1}{2}$ times for each rise of temperature by 10°, and the overall average of his own work, i.e. 2·499. Nor did he fail to point out that in inorganic chemical reactions also the Q_{10} increased as the temperature was lowered.

Subsequent workers found many cases in which embryonic growth followed the exponential rule. Erdmann & R. Hertwig gave figures for *Strongylocentrotus lividus*, from which Kanitz calculated temperature coefficients agreeing with Peter's. Bialascewicz confirmed the work of Hertwig on the amphibian embryo, but obtained a lower average temperature coefficient, namely, 2·40. For the early development of *Strongylocentrotus lividus* Loeb found in 1908 an average Q_{10} of 2·86, which varied thus with temperature:

°C.	Q_{10}
3–13	3·91
4–14	3·88
5–15	3·52
7–17	3·27
9–19	2·04
10–20	1·90
12–22	1·74

For the early development of *Ascaris megalocephalus*, Fauré-Fremiet found Q_{10}'s as follows:

°C.	Q_{10}
0–16	6·25
16–23	3·93
23–32	1·82

and for that of *Sabellaria alveolata*:

°C.	Q_{10}
7–13	2·66
13–16	2·16
16–19	1·28
19–22	1·27

Ephrussi (in 1926) studied the different phases of mitosis in sea-urchin and nematode eggs, allotting to each a characteristic temperature coefficient. Thus he obtained the following figures, working always between 18 and 25° C. and calculating for each period separately, not in a cumulative fashion from zero hour.

Table 70.

	Q_{10}	
	Strongylocentrotus lividus	*Ascaris megalocephala*
A (from zero hour to disappearance of nuclear membrane, zero being copulation of pronuclei in *S. lividus* and laying in *A. megalocephala*	2·31	2·20
B (from disappearance of nuclear membrane to the first appearance of the equatorial plate)	?	1·66
A plus *B*, i.e. the whole of the prophase	2·33	2·07
C (duration of the equatorial plate stage)	1·00	1·22
D (duration of the whole of the anaphase)	1·71	2·00
E (reconstitution of nuclei after mitosis)	1·39	1·33
A plus *B* plus *C* plus *D* plus *E*, i.e. the whole mitotic process... ...	1·70	1·80
B plus *C* plus *D* plus *E*, i.e. from disappearance of nuclear membrane to reconstitution of nuclei after mitosis	1·41	1·45

As Ephrussi says, the correspondence can hardly be a mere coincidence, and in any case the fact that change of temperature has almost no effect on process *C* must be significant.

We do not know whether the effect of increased temperature is greater on the mitotic or inter-mitotic period. Bucciante maintained chick embryo explants at 31° and at 41° and counted no more mitoses during a given amount of growth in the latter than in the former, so he concluded that the effect was the same.

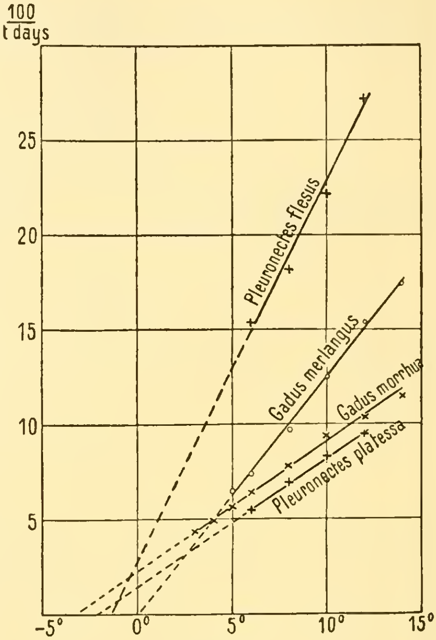

Fig. 78.

Loeb & Wasteneys found 2·3 for the embryonic growth of *Arbacia* and Bohr 2·9 for that of the snake *Coluber natrix*. Warburg gave 2·5 for the respiratory increase of *Arbacia* eggs. Herzog, working on the figures of Dannevig for the hatching time of the plaice egg (*Pleuronectes platessa*), got a Q_{10} of 2·5, on those of Earll for the cod egg (*Gadus morrhua*) 3·4 and 2·3, and on those of Ainsworth and Metzger for two different kinds of trout egg 3·4 and 5·3. Later, Bachrach & Cardot obtained a value of 3·2 for the embryonic growth of the slug *Agriolimax agrestis* between 6 and 23°, and one of 3·06 for that of the water snail *Limnaea stagnalis* between 11 and 32°. For *Ascaris megalocephala* Zavadovski got a Q_{10} of 2·8, but it was markedly higher at low temperatures than at higher ones. Then later, Zavadovski & Sidorov gave Q_{10} (18–28°) 3·12 for *Toxascaris limbata*, 3·12 for *Ascaris megalocephala* and 3·23 for *Ascaris suilla*, but 1·45, 1·46 and 0·73 respectively between 28 and 38°. Brown has also published a Q_{10} for *Ascaris*, Berrill for *Lophius americanus*, Harukawa for the oriental peach-moth, Kawajiri for *Salmo irideus*, Nakai for *Plecoglossus altivelis*, Kojiyama for *Pagrosoma major*.

But the older phase of the work on the effect of temperature on embryonic growth could not lead to any definite conclusions about the nature of the limiting factor or master process, in view of the fact that Q_{10} itself was not a satisfactory standard. When a physical process of diffusion (Nernst) could have a coefficient practically identical with a chemical process (saponification of ethyl butyrate by baryta, Trautz & Volkmann), both being about 1·3, it was difficult to say what might be producing a given Q_{10}. But more serious was the fact which soon became apparent, namely, that many cases of embryonic development could equally well be said to be simply linear functions of the temperature. Krogh was the first to find this. In considering the figures which had been obtained by Krogh & Johansen and by Dannevig on the rate of development of fish eggs and the hatching times at different temperatures, he found that the relation with velocity was a straight line and not an exponential curve, and that the relation with time was a curve which was not exponential but was a hyperbola. This is seen in Figs. 78 and

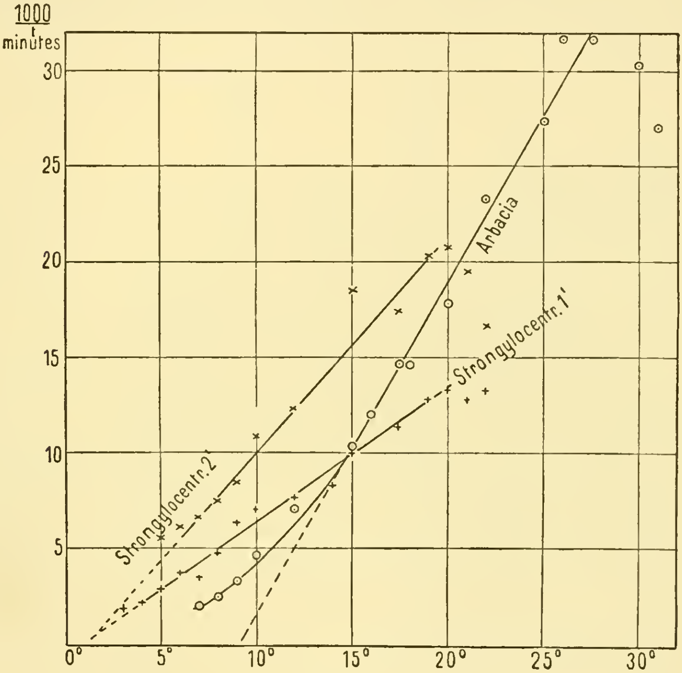

Fig. 79.

79, where the linear relationship clearly appears, and the velocity increment is directly proportional to the temperature increment. In 1913 he continued this line of thought by finding that the effect of temperature on (*a*) the rate of segmentation in the frog's egg, (*b*) the rate at which definite later developmental stages in the frog embryo are reached, and (*c*) the hatching time of a water-beetle, *Acilius sulcatus*, could in all these cases also be expressed by a straight line. In the case of the frog, as Krogh pointed out, it would have been absurd to express this linear relation in terms of Q_{10}, for Q_{10} would thus become:

°C.	Q_{10}
3–5	5·3
5–10	4·1
10–15	3·0
15–20	2·0

and could hardly be said to be a constant if it was constantly varying. Krogh did not compare his results with those of Hertwig, for the latter used rather different technique, e.g. transferring his embryos and eggs very slowly and by stages to the constant temperature basins. This procedure Krogh did not find necessary, though he observed that the limits of normal development are narrower during the earlier stages than they are later. The linear relationship thus found deviated, however, to some extent below 7°.

Exactly the same straight line was found when the velocities at which later developmental stages were attained were plotted against the temperature, though here divergence began as high as 12°. The hatching time of the water-beetle eggs behaved in the same way. Next Krogh took the figures of Loeb and Loeb & Wasteneys for echinoderm eggs already mentioned, and found that they also were best expressed by a straight line when $\frac{1000}{t \text{ in minutes}}$ was plotted against temperature. These results are all summed up in Table 71, which gives the geometrical characters of the straight lines, i.e. their slopes, together with other data about the embryos in question. In a further paper Krogh showed that the relation between the temperature and the carbon dioxide production of the chrysalides of *Tenebrio molitor*, the mealworm, could also be best expressed by a linear relation, and was not susceptible of description by the van't Hoff formula.

At this point the question became a matter of dispute concerning the proper way of calculating the results obtained in such experiments. Kanitz contested the truth of Krogh's view that the van't Hoff equation was inapplicable, on the grounds that the straight lines might be the result of two or three flat exponential curves. (Kanitz seems not to have avoided confusion between the time taken for development and the reciprocal of the time taken.) Kanitz plotted the log. of the velocity (log 1000/*t*) against the temperature, and obtained two straight lines in each case, just as Crozier did afterwards, with kinks between 10 and 15°. He assumed therefore that the Arrhenius formula could be used and calculated Q_{10} from it.

Table 71. *Effect of temperature on embryonic development.*

Krogh's figures:

Embryo	Process	Point where the line cuts the abscissa °C.	Velocity at 15° days	Limits of temperature between which linear relationship holds good	Limits of temperature between which development will go on at all	Investigator
Cod (*Gadus morrhua* and *aeglifinus*)	Fertilisation to hatching	−3·3	12·5	3·5–14	−1–14	Dannevig
Plaice (*Pleuronectes platessa*)	Do.	−2·1	11·6	6·5–12	—	,,
Cod (*Gadus merlangus*)	Do.	0	18·8	5·5–14	—	,,
Plaice (*Pleuronectes flesus*)	Do.	−1·4	30·3	6·5–12	—	,,
,, (*Pleuronectes platessa*)	Fertilisation to 4·9 mm. larva	−0·4	13·8	3·5–13	0–14	Johansen & Krogh
Sea-urchin (*Strongylocentrotus purpuratus*)	Cleavage 1	1·0	0·99	4–20	3·5–22	Loeb
	Cleavage 2	—	1·57	—	—	,,
Frog (*Rana butyrhina*)	Cleavage 1	2·7	0·511	7–21	3·5–22	Krogh
,, ,,	Later devel.	6·1	—	12–21	7·5–25	,,
Water-beetle (*Acilius sulcatus*)	Fertilisation to hatching	9·4	0·65	15–25	15–28	,,
Sea-urchin (*Arbacia*)	Cleavage 1	9·2	1·03	15–28	7–31	Loeb & Wasteneys

Krafka in 1919 found that in *Drosophila* the effect of temperature on rate of development could be expressed best by a straight line, but its effect on eye-facet number could be expressed best by an exponential curve. The appearance of both forms in one and the same living material made it unlikely that either relation needed correction. It was more probable that the formulae themselves were inadequate for proper analysis. Other lines of approach were made by entomologists on the one hand and by marine biologists on the other. Thus Reibisch worked over the original results of Dannevig, and concluded that a certain amount of heat was necessary for development and had to be supplied from outside, forgetting that

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no matter how hot the environment, the egg gets all its energy for development from the inside. Apstein, who noted with Krogh that the time/temperature relation was linear, i.e. the product was a constant, formulated another "day-degree theory", which, however, was no improvement. On the Reibisch-Apstein view, a certain sum of heat, a certain number of temperature units or day-degrees, is necessary to complete embryonic growth. Thus if an embryo hatches after 10 days at 12° 120 day-degrees would be said to have been required, so that at 6° 20 days would be required. Krogh & Johansen were easily able to show the superficiality of such a treatment.

Williamson's experiments with herring, haddock and plaice eggs also seem to show a linear time/temperature relation, but the variations are large. Elmhirst studied the hatching time of *Leander squilla* and the development of various decapod crustacea, and observed a constant time/temperature factor. He also went over the large number of fragmentary papers on the effect of temperature on the development of fishes which exist in the literature, mostly in Fishery Board Reports, and in many cases, though not in all, succeeded in finding evidences of the Krogh straight-line relationship between velocity and temperature. He thought that this emerged more clearly if 1·9° were added on to each temperature in order to have the freezing-point of sea water as a basis. But, on the other hand, in many cases, such as that of the cod (Fig. 80, taken from Fulton from various official Reports) the time/temperature curve could certainly be said to have a temperature coefficient (2·3 for 10–20° and 3·5 for 0–10°). Blunk working on *Dytiscus* embryos, and Blunk & Janisch working on those of *Blitophaga opaca,* obtained straight lines like Elmhirst for velocity/temperature graphs, i.e. hyperbolas for time/temperature graphs.

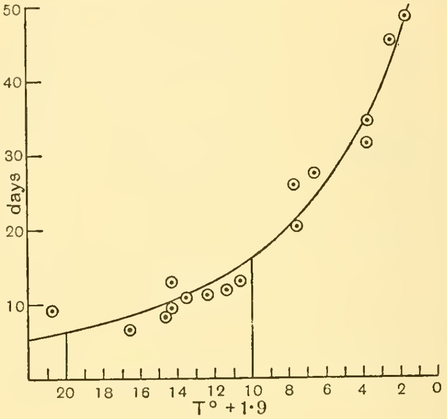


Fig. 80.

Sanderson has reviewed the work that has been done on the effect of temperature on the insect embryo, and has in many cases recalculated the figures of earlier observers, such as those of

Abbé: hatching of the Rocky mountain locust (*Melanoplus femurrubrum*).

Regener: hatching of the Pine bug (*Dendrolimus pini*).

Hennings: hatching of the Typographic beetle (*Tomicus typographus*).

Quaintaince & Brues: hatching of the bollworm (*Heliothis obsoleta*).

Girault: hatching of the bollworm (*Heliothis obsoleta*).

Jenne; Hunter & Glenn: hatching of the codling moth (*Carpocapsa pomonella*).

Jenne; Hunter & Glenn: hatching of a Braconid parasite (*Lysiphlebus tritici*) and of the green bug (*Toxoptera gramnium*).

Hunter & Hooker: hatching of the Texan cattle-fever tick (*Margaropus annulatus*).

All these data without exception, together with figures collected by Sanderson himself on the incubation of *Euproctis*, *Samia*, *Tenebrio*, *Leptinotarsa*, *Malacosoma*, etc., gave curves closely resembling those of Hertwig for the development of the frog. Thus the Q_{10} for Jenne's codling moth curve would work out at approximately 2·8 between 14 and 24°, and that for *Samia cecropia* would be 1·9 or so between 20 and 30°. In most cases the usual rise in temperature coefficient with decreasing temperature was shown, and in *Samia*, for instance, Q_{10} would be 2·5 between 10 and 20°. It is extraordinary that Sanderson made no calculation of temperature coefficients, but he was particularly interested in the practical application of his work and computed his results according to an empirical day-degree system which, as Martini showed, has no physico-chemical meaning, and even confuses intensity with quantity of heat. It is curious that ichthyologists on the one hand and entomologists on the other should have evolved very similar treatments of time/temperature curves both equally unsatisfactory. The temperature coefficients for these insect curves would work out somewhat as shown in Table 72. Peairs later tried to show that, for certain insects, the curve relating time taken in embryonic growth with temperature was a hyperbola, and he did indeed, over a short range, demonstrate the reciprocal to be a straight line, as, if that were the case, it should be. Peairs paid no more attention to the temperature coefficient question than did Sanderson. Precisely analogous experiments and results were obtained by Blunk working on *Dytiscus marginalis* and by Bodine on various grasshoppers.

Table 72.

Temperature coefficients of insect development.

Species	°C.	Q_{10}	Investigator
Browntail moth (*Euproctis chrysorrhea*)	16–26	2·0	Sanderson
Bombycid moth (*Samia cecropia*)	10–20	2·5	,,
,, ,,	20–30	1·9	,,
Mealworm (*Tenebrio molitor*)	10–20	3·8	,,
,, ,,	20–30	2·1	,,
Colorado potato-beetle (*Leptinotarsa decemlineata*)	10–20	2·5	,,
,, ,, ,,	20–30	2·0	,,
,, ,, ,,	20–30	2·2	Girault
,, ,, ,,	20–30	2·0	Girault & Rosenfeld
Pine bug (*Dendrolimus pini*)	15–25	1·4	Regener
Typographic beetle (*Tomicus typographus*)	15–25	2·9	Hennings
Bollworm (*Heliothis obsoleta*)	10–20	4·4	Quaintaince & Brues
,, ,,	20–30	2·0	,, ,,
Codling moth (*Carpocapsa pomonella*)	15–25	2·8	Jenne
Braconid parasite (*Lysiphlebus tritici*)	0–10	2·3	Hunter & Glenn
,, ,, ,,	10–20	3·3	,, ,,
Green bug (*Toxoptera gramnium*)	0–10	2·5	,, ,,
,, ,,	10–20	3·3	,, ,,
Texan cattle-fever tick (*Margaropus annulatus*)	10–20	3·1	Hunter & Hooker
,, ,, ,,	20–30	2·1	,, ,,
,, ,, ,,	30–40	1·8	,, ,,
Codling moth (*Carpocapsa pomonella*)	15–25	2·7	Hammar
Gypsy moth (*Porthetria dispar*)	5–15	4·9	Peairs
,, ,,	15–25	1·9	,,
Locust (*Melanoplus differentialis*)	23–33	2·5	Bodine
,, (*Melanoplus femur-rubrum*)	23–33	2·4	,,
Green-striped locust (*Chortophaga viridifasciata*)	23–33	2·8	,,

Bachmetiev made some experiments like those of Sanderson and Peairs, and Kanitz reviewed all the older literature in 1915. Kanitz also suggested that the variations found in the pregnancy time of mammals, and especially man, might be related to the differing body-temperature in individuals, for 2° at a Q_{10} of 2·5 would in man make a difference of 10 days. This notion was supported by O. Wellmann, who went through the statistics of pregnancy time in mares and found that, while the average was 326 days from July to September, it was 343 days from March to May. Since the researches of Cobelli; Vos; Congdon; and Sumner have all shown that the body-temperature is raised to a slight degree when the environing atmosphere is warm, the gestation time might very well be expected to show the seasonal rise and fall which it actually does. Vicarelli's thermometric observations support Kanitz's theory.

2·16. Temperature Characteristics

The older phase of the subject ended, then, in a rather barren doubt as to whether the van't Hoff equation was applicable to living processes such as those of the growing embryo, or more correctly whether the time/temperature relation was best expressed by a curve of exponential form or by a hyperbola. In so far as it was applicable, it gave definite information that the limiting factor of embryonic growth was probably chemical rather than physical, but that was all. Snyder's paper of 1908 introduced a new period, that of the use of the Arrhenius equation. This expression is

$$K_2 = K_1 \, e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \quad \text{or} \quad \frac{K_2}{K_1} = e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)},$$

where K_2 and K_1 are the velocity constants of the reaction in question at the high and low temperatures, T_2 and T_1, chosen respectively, e the base of Napierian logarithms, 2 the gas constant, and μ the gram molecular energy of activation of the catalyst, i.e. the "critical increment of the active substance" if the reaction is monomolecular, or the sum of the gram molecular energies of the substances if the reaction is bimolecular. The temperature is expressed in degrees absolute. If the velocity constants cannot be calculated, the reciprocals of the time taken to do a definite amount of embryo formation or other work may be used instead, so that the relation becomes:

$$\frac{\frac{1}{g_1}}{\frac{1}{g_2}} = e^{\frac{\mu}{2}\left(\frac{T_1 - T_2}{T_1 T_2}\right)},$$

where g_1 and g_2 are the times in question, e.g. 10 days from fertilisation to hatching at one temperature, 20 days at another. When

$$T_2 - T_1 = 10, \text{ then } \frac{K_2}{K_1} = Q_{10}$$

of the van't Hoff equation. The relation may also be expressed:

$$\mu = 4{\cdot}61 \, \frac{\log K_2 - \log K_1}{\frac{1}{T_1} - \frac{1}{T_2}}.$$

It was originally suggested by Arrhenius as an empirical description of the facts, but it now has through the work of Rice; Rodebush;

and Thomson, a solid theoretical basis. The quantity μ is called the temperature characteristic in distinction from the temperature coefficient, for it is the calculated "energy of activation" of the active molecule in the reaction governing the slowest process in the complex chain of processes under investigation. It is an index, therefore, of the nature of the catalyst in operation, and should do much more than merely distinguish between chemical and physical processes. Reactions having the same catalyst have the same temperature characteristic. For chemical reactions μ varies between 4000 and 35,000, and can be represented graphically by the slope of the straight line or lines, if there is a break, relating the log. of the velocity in question to the reciprocal of the absolute temperature. The break indicates that one reaction of the catenary has ceased to be the slowest and another has taken its place. The steeper the slope, the greater the increase of velocity with unit rise, and the higher the value of μ. The log. effect/reciprocal of absolute temperature relation does not always give a straight line, but only if the process that is being measured is irreversible. If an equilibrium effect is functioning, then the relation will be a curve. Finally, μ has nothing to do with the Q of the van't Hoff equation, for the former has reference to heat of activation and the latter to heat of reaction.

The advantages of the Arrhenius equation over the van't Hoff equation are considerable. The former has a much greater range of values dealing with thousands instead of decimal units. The Arrhenius equation also reveals abrupt breaks in the straight lines relating log. effect to the reciprocal of absolute temperature at which one limiting reaction is supposed to take the place of another; these are masked by the van't Hoff equation. Then Q_{10} is not a constant while μ is, at any rate between certain definite temperatures where the breaks occur. But most important of all, Q_{10} values cannot be definitely associated with specific types of chemical reaction, such as oxidation-reduction, hydrolysis, and synthesis, so that all one can hope to find out by the van't Hoff equation is whether the process is physical or chemical, while with the Arrhenius equation one may discover, perhaps, of what nature the controlling change is at any given moment.

Arrhenius himself in 1915 made many applications of this equation to biological processes, and it is interesting that he gives as an average value for segmenting eggs μ 14,100, but without any reference or even any intimation whether this was based on results obtained by himself or by others. The matter is curious, for 14,000 is, as will be seen, not now regarded as a zone occupied by growth processes. But the equation was first applied in physiology by Snyder, who calculated μ for various temperature/time effects in muscle action, and compared them with others from various sources. Thus he found a μ of 12,800 calories for the experiments of Peter with echinoderm egg-cleavage and of 16,600 for those of Hertwig on amphibian egg-cleavage. He did not, however, carry the question much further, and it was left for Crozier and his collaborators, in a long series of papers, to work out the temperature characteristic for a great number of processes. Before discussing the findings in the case of embryonic growth, we must outline the groups into which Crozier found he could separate living processes as regards their temperature characteristic.

The temperature characteristic of many respiration processes, both *in vivo*, such as the oxygen consumption of *Arbacia* eggs, and *in vitro*, such as the combination of oxygen with haemoglobin, was about 16,600. In many cases an abrupt break in the straight lines would occur; thus below 15° μ for the oxygen consumption and ciliary activity of mussel gills was 16,000 and above 15° it was 11,000, which latter value held at all temperatures tried for the oxygen consumption of the guinea-pig uterus. On the other hand, the rate of progression of all kinds of invertebrates usually had a μ of between 12,000 and 12,500, and such phenomena as the frequency of the firefly flash, the frequency of invertebrate heart beats, and the frequency of noise production also came in this group. A μ of 12,000 would seem to indicate the predominance of a nervous factor, and of 16,000 the predominance of an oxidation. Other processes gave various values of μ, e.g. 9240 for rate of movement of algae, from 4700 to 10,300 for protoplasmic streaming in plant cells, and 7900 for rate of pulsation of infusorial contractile vacuoles. These would possibly indicate the appearance of a physical factor, probably diffusion. Rate of nerve conduction gave 8080 and 10,700. It is probable that even a difference between 16,000 and 16,700 is significant, for the former may indicate that the controlling catalyst in the oxidation is iron, while the latter may indicate that a dehydrogenation mechanism is at work; again, the value 11,500, which some oxidations show at some temperatures, is closely associated with hydroxyl ion catalysis. Glaser suggested that the value of 8000 which he found for a phase of *Paramoecium* locomotion was associated with hydrolysis. Crozier & Stier found that the μ for various processes in the grasshopper was quite different after decapitation to what it had been before, changing from 7900 to 16,500, and showing that, after central nervous system control had been removed, an oxidation was the slowest reaction. "The velocities of vital processes", said Crozier, "are determined by the velocities of dynamically linked systems of chemical transformation differentially affected by external conditions." Table 73 gives a résumé of the commonest μ values and their significances.

Table 73.

	Significance	
μ	Physico-chemical	Biological
4,700 8,000 9,000 10,000	Hydrolysis or diffusion	Protoplasmic streaming Rate of nerve conduction Rate of moving algae
11,300	Hydroxyl ion catalysis of oxidations	Oxygen consumption
12,000 12,500	Breaking of 1 sulphur linkage	Nervous factor
16,000 16,600	Catalysis by iron dehydrogenation	Oxidation, oxygen consumption
18,000	Activation of iodine	—
20,000	Hydrogen ion catalysis	—
24,000	Certain hydrolyses	—
27,000	—	Growth

In 1926 Crozier marshalled all the values that had been obtained for μ in biological processes into a frequency polygon, from which it appeared that the commonest types of μ were roughly 8000, 11,000 and 16,000; above that the peaks or modes were not so striking, for the observations were fewer. He also marshalled all the values that had been obtained for breaks in temperature characteristics at definite temperatures into another frequency polygon, from which it appeared that 15° was by far the most important of these, but that 8°, 20° and 30° were also associated to some extent with abrupt breaks in the values of μ. Less common were breaks at 4·5°, 25° and 27°. That both these polygons are definitely multimodal is a fact which makes the whole position more convincing than it might have been; for, if chance had been operating alone, there would have probably been but one peak.

These preliminaries concluded, one can examine the results of Crozier for growth in general and embryonic growth in particular. He found on examining the figures for the growth at different temperatures of *Drosophila melanogaster* embryos (and larvae) (Loeb & Northrop and Krafka) and of the pupae of *Tenebrio molitor* (Krogh) that there were two temperature characteristics, 27,000 below 15° and about 10,000 above 15°. This was evidence that whatever the limiting factor in growth was, it was not an oxidation process, a conclusion that was strongly supported by the data of Loeb & Wasteneys and Loeb & Chamberlain for the velocity of segmentation (first cleavage) of echinoderm eggs. This worked out at 41,000 below about 11°, 21,000 between 11° and 16°, and 12,400 above 16°. Krogh's data, again, for the segmentation of frog's eggs, gave 22,600 below 13·5° and 10,200 above 13·5°. In no case, therefore, was a typical oxidation temperature characteristic obtained for the earliest stages of embryonic growth.

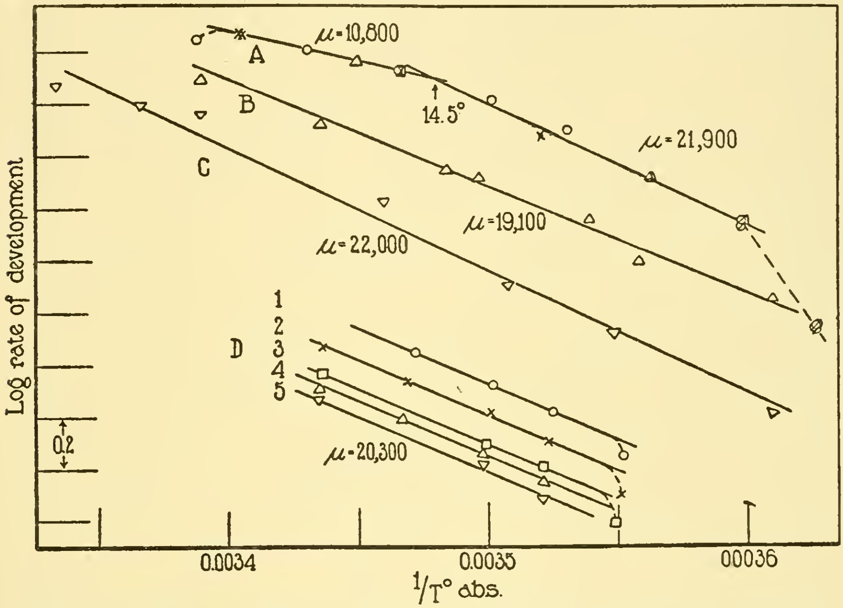


Fig. 81. Temperature characteristics of early development in the frog. *A*, Krogh's data (first cleavage); *B*, Lillie & Knowlton's data (from first cleavage to disappearance of yolk-plug); *C*, Lillie & Knowlton's data (from other cleavages to disappearance of yolk-plug); *D*, Krogh's data (later development).

Crozier himself emphasised the view that these facts opposed the theory of metabolic gradients (see Section 3·8), and, in so far as that theory holds that such tissues as have the highest metabolic rate (respiration intensity) must also be growing the fastest, Crozier's facts do oppose it. Other work on the temperature characteristics of growth confirmed that of Crozier; Brown, for instance, found μ's of 28,000, 17,210, 7410 and 19,800 for the adult development of cladoceran arthropods, though in one instance it must be admitted that he got a value of 16,950 (*Simocephalus serrulatus*).

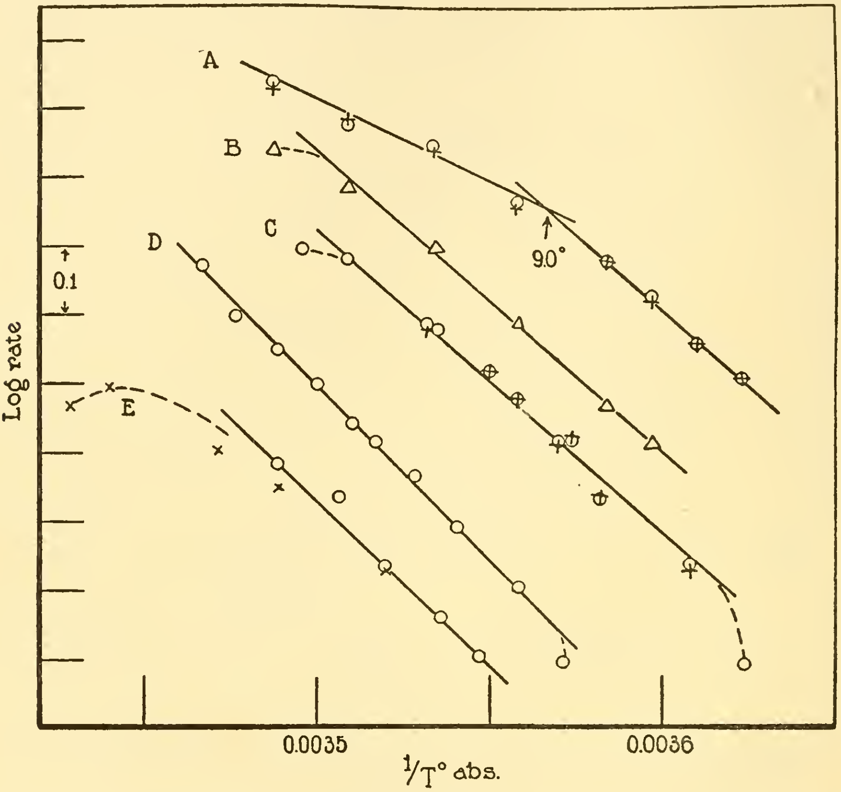


Fig. 82. Temperature characteristics of early development in teleostean fishes. *A*, *B*, Dannevig's data (*Gadus*); *C*, Krogh & Johansen's data (*Pleuronectes*); *D*, Higurashi & Tauti's data (*Hypomesus*); *E*, Higurashi & Nakai's data (*Plecoglossus*).

In a later paper, Crozier considered fully the results of many other investigations on the effect of temperature on embryonic growth. Figs. 81 and 82 show some of the results. Fig. 81 includes the data of Krogh; Lillie & Knowlton; and Hertwig on early development in the frog, and Fig. 82 includes the data of Dannevig; Krogh & Johansen; Higurashi & Tauti, and Higurashi & Nakai on development in various teleost fishes. The regularity with which values are obtained for μ of about 20,000 is remarkable; thus the first cleavage of *Rana* eggs has 21,900, the third cleavage to yolk-plug disappearance in *Rana* has 22,000, the total incubation time of *Hypomesus olidus* has 23,700 and of *Plecoglossus altivelis* 23,000. Blunk's figures for the rate of development of *Dytiscus marginalis* give 19,300 and of *Dytiscus semisulcatus* 20,000. Ziegelmayer's figures for the rate of development of *Cyclops* give 15,700, which is on the low side. Bliss's figures for *Drosophila* give a very similar result. The fact that the same temperature characteristic holds good for various different periods during one continuous developmental process obviously suggests that the shape of the curve must be much the same at different temperatures. The developmental process as a whole is therefore not deformed by change of temperature, but simply lengthened or shortened, its shape, as it were, remaining the same, or, in other words, either only one velocity constant is involved, or else, if more than one, velocity constants of the same temperature characteristic. To what extent this is really the case will appear presently.

The only work on the temperature characteristic of the development of the chick is that of Brody & Henderson, who studied the effect of incubation at different temperatures upon the growth-rate of the embryo. Fig. 83 *a* taken from their paper shows how at any given day during development the weight attained is greater the higher the temperature; thus at the 17th day the average weight at 35·0° C. is 5·510 gm., at 37·3° 12·685 gm. and at 40·5° 20·427 gm. The upper part of the same graph shows the log. weight plotted against the age, as is usual in Brody's method, and the slopes of the resulting series of straight lines give the instantaneous percentage growth-rates, as follows:

	Phase or cycle		
	1st	2nd	3rd
°C.	(*k*)	(*k*)	(*k*)
40·5	70	38	20
37·3	56	36	24
35·0	62	34	20
34·4	46	22	—

The instantaneous percentage growth-rate is shown in Fig. 83*b* related to the temperature. The effect of temperature on the earliest phase of growth is obviously the greatest of the three; in other words, in the earlier stages rise in temperature has a proportionally greater effect on the instantaneous percentage growth-rate than in the later stages. In the intermediate stage the rise is not so steep

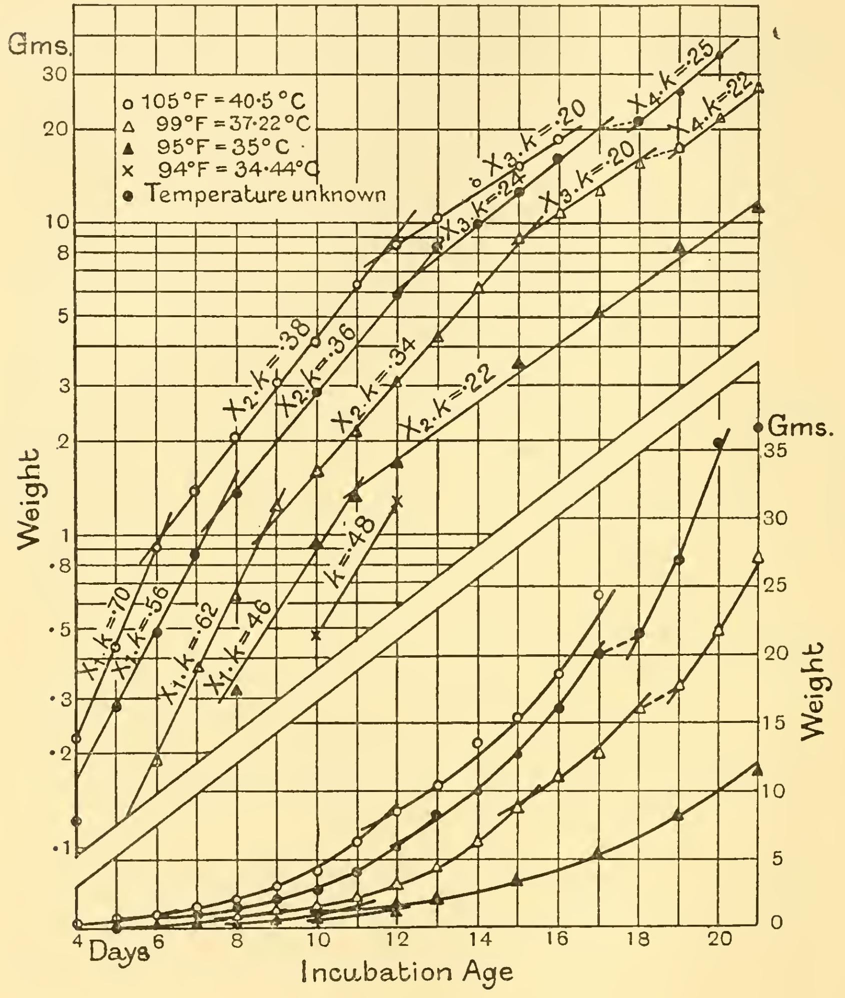


Fig. 83*a*.

with rise of temperature, and in the last stage, i.e. from about the 14th day onwards, the effect is nil, for the line is horizontal; or, in other words, the heat-regulating mechanism in the embryo is sufficiently developed at this stage to enable it to keep its body-temperature constant within the limits of the experimental temperatures so that they do not get a chance of affecting its growth-processes. It could indeed be argued that the three curves show a progressively increasing command of body-temperature, so that higher temperature coefficients and steeper growth-rate/age curves are inevitably found the younger the embryo is. This question of temperature-regulation will be taken up again later in the book (see Section 4·21).

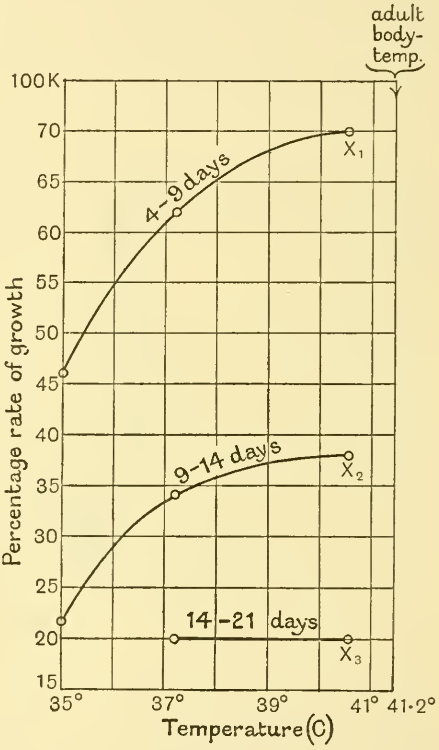


Fig. 83 *b*.

From these data the μ constants of Arrhenius' equation was calculated. For phase 1 it was 25,700 below 37° and 7100 above it, while for phase 2 it was 39,050 below 37° and 6500 above it. These values agree well with those obtained for other instances of embryonic growth, a fact which is especially important in view of the restriction of the other data to amphibia, fishes and arthropods. For the third phase there was obviously no temperature characteristic. Thus, before the age of about 13 days and between the temperatures of 35 and 37° increase of temperature by ten degrees more than doubles but does not quite triple the rate of growth, while after the 14th day the growth of the embryo is practically unaffected by change of temperature owing to its acquired homoiothermicity.

Taking the values which have been obtained for μ for growth and development, it is clear that they all cluster rather closely round certain definite magnitudes. Fig. 84, constructed from the data of all the relevant investigations, shows a frequency polygon for the values of μ associated with growth. Crozier's polygon for all μ values (286 investigations) is described by a continuous line, and demonstrates the well-known modes at 8000, 11,000 and 16,000, as well as the smaller ones at 20,000 and above. These include all biological reactions which have been studied at different temperatures. But when only such μ values as have been obtained for growth are

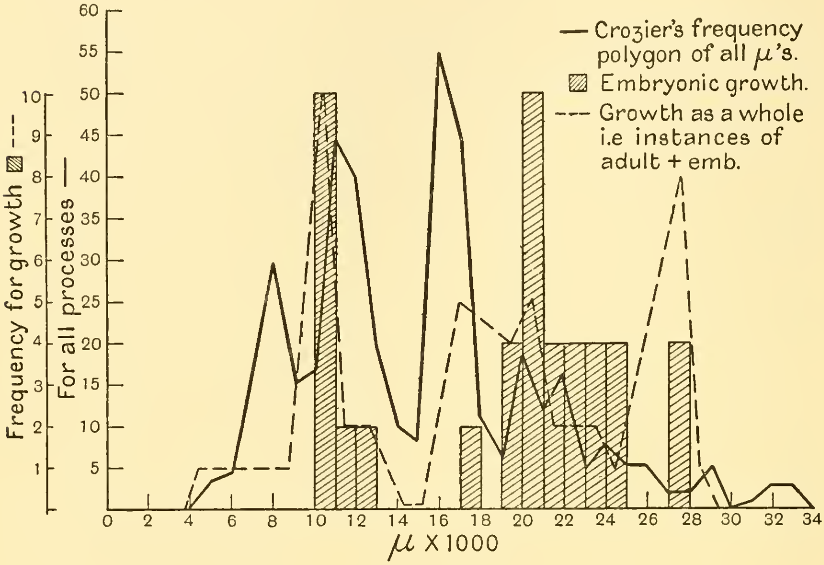


Fig. 84.

plotted, the picture is quite a different one, for the modes do not correspond at all with those of the wider series. For the growth-process, they exist at about 10,000, from 17,000 to 21,000 and at 27,000. The first of them falls between two peaks of Crozier's polygon, and the second, though represented there, equals, in this narrower consideration, the great 16,000 oxidation mode. The growth-frequency polygon obviously has little to do with oxidations, which are here graphically seen not to be the limiting factors in the growth-process. If now embryonic growth alone is considered, an even narrower situation is disclosed, for embryonic growth has a peak at 10,000, practically nothing at all between 13,000 and 19,000, and after that a sustained high series until 25,000 is reached, followed by a spurt at 27,000. In a word, the peaks of growth as a whole and embryonic growth are very much alike, namely at 10,000, 20,000 and 27,000, but the middle one of these is specially associated with the development of the embryo. Now there can be no doubt but that the velocity of growth (reciprocal of the time required to attain a given size or stage of development) can be expressed well in its relation to temperature by the Arrhenius equation. Unfortunately, there is difficulty in deciding what is the exact meaning of the frequency with which the value of 20,000 occurs. There seems to be no reason why this should be so usual a feature of the time/temperature relation in embryos, and the modes at 10,000 and 27,000 are equally mysterious. No doubt it may be expected that further work, both in the direction of precision of data and in the direction of explanation of the significance of these values, will clear the matter up. The identification of the reactions responsible would certainly be a desirable thing. At present there is only one suggestion as to the meaning of a μ of 20,000, namely, its association by Glaser with "mobilisation hydrolysis", i.e. the production of oxidisable substances by hydrolytic action such as the formation of glucose from glycogen. The mode at 10,000 might be ascribed to synthetic processes, but this would apply better to the growth-values of 7000 or thereabouts. "I am quite unable to attach possible specific significance to temperature characteristics 8000 and 27,000", says Crozier in a private communication. "It will be realised that in the case of the earlier statements one is dealing with somewhat uncertain suggestions of which the chief value may be that they serve to suggest specific experiments. I should personally tend to deplore theories of the physico-chemical control of differentiation and development which might very easily be erected on the basis of such speculation." In these circumstances all that can be done is to note the values of μ which have been so far obtained for embryonic growth without attempting to give them any meaning.

Perhaps the most important result of this kind of work is that no embryonic growth-process so far investigated turns out to have a temperature characteristic of 16,000, which is the typical one for oxidations. In this connection the paper of Loeb & Wasteneys in 1911 is interesting; it was entitled: "Are oxidation processes the independent variables in life phenomena?" It occurred to them to

Table 74. *Results of investigations of temperature characteristics.*

Species	Growth	Embryonic growth	Investigator
Mealworm (*Tenebrio molitor*) (pupa)	27,000	—	Krogh
,, ,, ,,	10,000	—	,,
Fruit-fly (*Drosophila*) (egg + larva + pupa)	27,000	27,000	Krafka
,, ,, ,,	10,000	10,000	,,
,, ,, (imago)	19,920	—	Loeb & Northrop
,, ,, (egg + larva)	27,000	27,000	,, ,,
,, ,, ,,	10,000	10,000	,, ,,
,, ,, (pupa)	27,000	—	,, ,,
,, ,, ,,	10,000	—	,, ,,
Sea-urchin (*Arbacia*) (segmentation)	—	41,000	Loeb & Wasteneys
,, ,,	—	21,000	Loeb & Chamberlain
Frog (*Rana*) (segmentation)	—	12,400	Krogh
,, ,,	—	21,900	,,
,, ,,	—	10,800	,,
Frog (*Rana*) (1st segmentation to disappearance of yolk-plug)	—	19,100	Lillie & Knowlton
Frog (*Rana*) (3rd segmentation to disappearance of yolk-plug)	—	22,000	,, ,,
Frog (*Rana*) (later)	—	20,300	Krogh
,, (till hatching)	—	20,000	Barthélemy & Bonnet
,, (early)	—	17,000	Hertwig
,, (later)	—	24,000	Lillie & Knowlton
Cod (*Gadus morrhua* and *aeglifinus*)	—	11,800	Dannevig
,, (*Gadus merlangus*)	—	20,000	,,
Plaice (*Pleuronectes platessa*)	—	20,000	Johansen & Krogh
Japanese teleost (*Hypomesus olidus*)	—	23,700	Higurashi & Tauti
,, ,, (*Plecoglossus altivelis*)	—	23,000	Higurashi & Nakai
Water-beetle (*Dytiscus marginalis*)	—	19,300	Blunk
,, (*Dytiscus semisulcatus*)	—	20,000	,,
Water-flea (*Cyclops*)	—	10,400	Ziegelmayer
,, ,,	—	15,700	,,
Fruit-fly (*Drosophila melanogaster*)	33,210	—	Bliss
,, ,,	16,850	—	,,
,, ,,	7,100	—	,,
Water-flea (*Pseudosida bidentata*)	19,800	—	Brown
,, (*Moina macrocopa*)	28,500	—	,,
,, ,,	17,210	—	,,
,, ,,	7,410	—	,,
,, (*Simocephalus semisulcatus*)	16,950	—	,,
,, ,,	4,780	—	,,
Hen (*Gallus domesticus*)	—	25,700	Brody & Henderson
,, ,,	—	7,100	,, ,,
,, ,,	—	39,050	,, ,,
,, ,,	—	6,500	,, ,,
Fruit-fly (*Drosophila melanogaster*)	27,800	—	Plunkett
,, ,,	17,100	—	,,
,, ,,	9,000	—	,,
Trout (*Salmo fario*)	—	24,500	Gray
,, ,,	—	20,000	Maitland

Unless otherwise stated the lower μ is above 15°.

compare the temperature coefficient of embryonic development in the sea-urchin's egg with the temperature coefficient of respiratory rate in the same material. Their figures for developmental rate gave very considerable slowing at the low temperatures, so that although the coefficient between 17 and 27° was 2·6, between 8 and 18° it was 6·0. The temperature coefficient for the respiratory rate, however, did not show this, and, while it was 1·5 approximately round 30°, never rose higher than 2·45 even including the data for between 3 and 13°. There was thus no correspondence between the two sets of coefficients below 15°. This circumstance, they thought, harmonised with the fact that potassium cyanide in concentrations which stopped development altogether still permitted 25 per cent. of the oxidations to proceed, and further, with the fact that when no development was going on normally, i.e. before fertilisation, there was still a certain respiration. Of the two processes the morphological development rate seemed to be the more easily abolished by external agents. But above 32° when cleavage became impossible, respiration was also impossible, and the oxygen uptake rapidly fell off. "These facts", said Loeb & Wasteneys, "do not contradict the view that oxidations are the independent variables in developmental processes. But on the other hand they do not furnish any convincing evidence in favour of such a view." The whole incident, as a matter of fact, may now be regarded as an instance of the incapacity of the ordinary van't Hoff equation to differentiate between two alternatives in a complicated biological system, and the question which Loeb & Wasteneys were trying to answer may be said to be solved by the frequency polygon of temperature characteristics.

The results of all this work may be summed up by saying that by the use of the heat effect on embryonic growth and development a kind of dissection has been made of the process, for at one temperature one controlling reaction or pacemaker is laid bare, as it were, and, at another temperature, another controlling reaction. This exposing method has led to the unveiling of four or five reactions of quite clear-cut temperature characteristics, which according to the temperature and the circumstances may be in control of embryonic growth. What these reactions are we do not yet know, but admittedly a good step forward has been taken by the identification of the heats of activation of their active molecules.

Crozier has discussed in some detail the relation between the temperature characteristic of growth and the autocatakinetic growth curve. Difficulties arose out of the experimental fact that temporary alteration of the temperature during a period of development modified the temperature characteristic for the remainder of development. This it could not be expected to do on the Robertsonian view, for where there is only one velocity constant, as in his presentation, its temperature characteristic must always be the same, and changing the temperature would only multiply the time coordinate by some constant, and drag out or compress the time taken to complete the autocatalytic curve. Within one cycle the temperature characteristics should be the same for all partial developmental periods as well as for the whole. This, however, was found not to be the case by Bliss for *Drosophila*, by Brown for the cladoceran *Pseudosida bidentata*, and by Titschak for the clothes-moth *Tineola biselliela*. If an animal is allowed to develop for 50 per cent. of the total normal time at 15° C., and is then transferred to a temperature of 25° C., it may take less or more time to finish its development than would be predicted on the basis of the fact that it has still 50 per cent. of its normal time to go at 15°. Brown expressed the differences as per cent. gain or loss in time under such treatment, and found that they were, though small, quite significant statistically. This means that the growth equation of Robertson,

$$\frac{dx}{dt} = Kx\,(a - x)$$

(where a is the initial amount of growth-forming substance, x the amount formed after time t and K a constant), cannot hold, and must give place to an equation with two velocity constants. Crozier has suggested one, the differential form of which is

$$\frac{dx}{dt} = (K_1 + K_2 x)\,(A - x),$$

where K_1 is the velocity constant proper to the reaction $A \rightarrow x$, but in the absence of the catalytic effect of x, while K_2 is the velocity constant associated with the process when x is functioning as a catalyst. The point of inflection will then be

$$x = \frac{K_2 A - K_1}{2K_2}.$$

The integral form of the differential equation is

$$t = \frac{1}{K_1 + K_2 A} \log \frac{A\,(K_2 x + K_1)}{K_1\,(A - x)},$$

and though the form of its curve is sigmoid the point of inflection depends on the ratio of the two velocity constants, so that it may be very asymmetrical.

Before embarking on the exposition of the applications of the Arrhenius formula to growth in general and embryonic growth in particular, it was said that there was much difference of opinion as to whether the time/temperature relation could be expressed best by a hyperbola or an exponential curve. It was often pointed out by Loeb and others that even a straight line might be produced by the action of various factors on a true exponential curve causing it to flatten out. Snyder suggested that an important factor which might be expected to have such an effect was the protoplasmic viscosity. This idea has since had a considerable popularity, although Crozier has vigorously combated it. "The attempt to introduce considerations of protoplasmic fluidity, presumably as influencing diffusion, requires a theory of the general control of organic activities by the whole body of the cell rather than at surfaces. This is unnecessary", says Crozier, "and at present inadmissible." But it is by no means easy to see why, and the possibilities contained in corrections for viscosity should certainly be explored. The difficulty is that viscosity varies in different ways with temperature according to the animal or plant used. Some authors, moreover, seem to wish to obtain a constant Q_{10}, although even in inorganic *in vitro* reactions Q_{10} is never constant, but increases as the temperature is lowered. Pantin, for instance, corrected various temperature coefficients, on the basis of the viscosity changes in *Nereis* eggs, and found a much greater approach to constancy, though even after correction, the Q_{10} rose a good deal at the lower temperatures. Krafka's work, already referred to, is rather an obstacle to this point of view.

The same line of thought was carried further still by Bělehrádek, who criticised the use of the Arrhenius equation on the ground that, like the van't Hoff formula, it also did not give a constant for all temperatures at which normal development will proceed, but shows up critical points which may or may not be real. This criticism was also made by Heilbrunn. Bělehrádek used a modification of the Esson-Harcourt equation, which he found would represent the time/temperature relation fairly accurately:

$$y = \frac{a}{x^b} \quad \text{or} \quad \log y = \log a - b \log x,$$

where y is the time necessary to accomplish a given amount of e.g. embryonic development at the temperature x and a and b are constants. Thus log. time/log. temperature gives a straight line. The constant a varies according to the unit of time used, but the constant b is independent of the time-unit and the actual velocity for it represents acceleration. If $b = 1$ then $y = a/x$, which is the equation for a rectangular hyperbola, and covers the work of Peairs on insect development as a special case of the general law. Again, the straight-line relation of Krogh and others is another special case, for, according to it, $v = kx$ where v is the velocity and x the temperature and, as $y = 1/v$ and $k = 1/a$, we get $y = a/x$, which is the original equation once more. As b is usually greater than 1, Q_{10} usually declines with rise of temperature. Bělehrádek regards the constant b as a true temperature coefficient, for it does not change with temperature, and he has calculated it for various systems which are relevant here. Thus he gets for the embryonic development of

	b
Copepod *Cyclops fuscus* (Ziegelmayer)	1·16
Fruit-fly *Drosophila melanogaster* (Loeb & Northrop) ...	2·10
Frog *Rana virescens* (Lillie & Knowlton)	2·36
Hen *Gallus domesticus* (Féré)	4·10

and for the early segmentation of

	1st	2nd
Sea-urchin *Strongylocentrotus lividus* (Loeb)	0·99	1·13
Roundworm *Ascaris megalocephalus* (Fauré-Fremiet) ...	2·50	—

It would appear that b increases with age, for

Fruit-fly *Drosophila melanogaster* (Loeb & Northrop):	*b*
Larva	2·10
Pupa	2·28

and

Water-beetle *Dytiscus semisulcatus* (Blunk):	
Embryo	1·10
Larva (1st instar)	1·14
Larva (2nd instar)	1·26
Larva (3rd instar)	1·38
Prepupa	1·48
Pupa	1·60

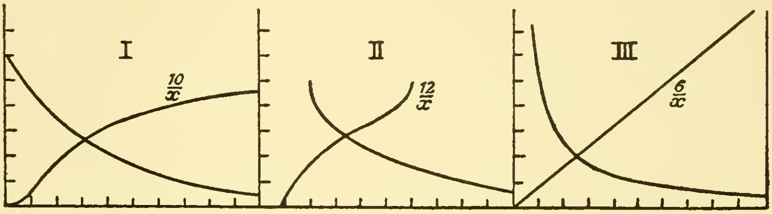
and

Frog *Rana fusca* (Krogh):	
Fertilisation to 1st cleavage	1·20
End of 1st cleavage to closure of neural fold ...	1·64
Closure of neural fold to appearance of external gills	1·76
Appearance of external gills to 3-branched gills	1·92
3-branched gills to 7 mm. embryo	1·69
7 mm. embryo to 7·8 mm. embryo	2·52

and

Typographic beetle *Tomicus typographus* (Hennings):	
Embryo	2·02
Larva	3·52

Bĕlehrádek interprets *b* as being a measure of protoplasmic viscosity, so that in his opinion all temperature effects on growth can be regarded as primarily effects upon viscosity, and only acting indirectly upon the rapidity of the growth-process. Whether this point of view will prove to be either more fruitful or more correct than that of Crozier and his collaborators cannot at present be decided. Crozier & Stier have, however, shown that the fit of Bĕlehrádek's formula in one case, at any rate, is not at all good, while Bĕlehrádek has strengthened his case by studying the time factor in cooling and heating protoplasm. But it is interesting that the constant *b* should increase with age, for protoplasmic viscosity almost certainly does, and this has an obvious importance in view of the gradual loading up of the cells of the body with paraplasmatic substances. Bĕlehrádek has answered Crozier's criticisms and the papers must be consulted for the details of the argument.



Exponential curve Catenary Hyperbola

Fig. 85.

Janisch represents the time/temperature relation not by a simple exponential curve, nor by a hyperbola, but by a "Kettenlinie" or catenary curve. The reciprocal of this is not a straight line or a sigmoid curve rising most rapidly at the point of inflection; it is a sigmoid curve rising most slowly at the point of inflection. Such a complex exponential curve should therefore be produced when the velocity/temperature graph is drawn, and Janisch actually showed that Krogh's straight lines turn into curves of this character when the points which Krogh neglected at the upper and lower ends are taken into consideration. Just the same can be shown for the figures of Sanderson and Peairs. Fig. 85, which is taken from Janisch, shows the relations between these curves. Fig. 86, also taken from Janisch, shows a replotting of the data of Sanderson for *Margaropus annulatus*. The time of development is shortest at 28°, and, just as Fauré-

Fremiet showed for the segmentation of *Ascaris*, it lengthens a little on the high-temperature side of that. The points are well described by one of Janisch's catenary curves. The velocity of development, i.e. the reciprocal of the development time, correspondingly rises to a maximum at 28°, and has a sigmoid form on each side of this maximum. This peaked curve, part of which, it should be remembered, corresponds to the Krogh "straight-line", is identified by Janisch with the peaked curve which Duclaux in 1899 pointed out would be produced by the operation of two separate factors giving complex exponential curves, i.e. differentially affected by temperature change. This peaked curve could be either symmetrical or asymmetrical.

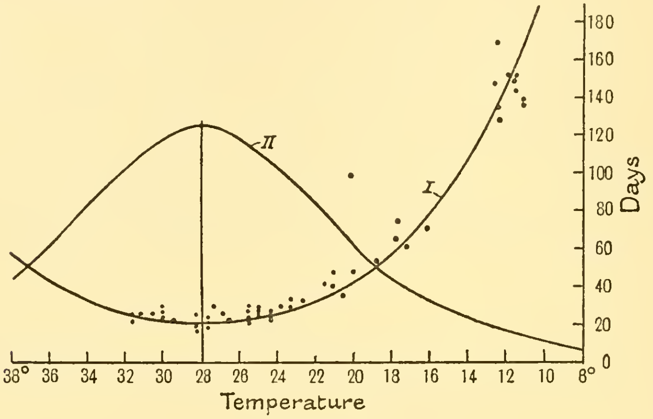


Fig. 86.

Perhaps further work along these lines will lead to conclusions about critical temperatures and controlling reactions which would support those of Crozier.

Janisch gave a full mathematical treatment of these questions, and also reported an investigation of the effect of heat on the embryonic development of the "Mediterranean flour-moth" *Ephestia kühniella* (see also Hase). The time/temperature relation here also can be expressed by an equation of the form

$$y = \frac{m}{2}\,(a_1^{x} + a_2^{-x}),$$

where m and a are constants and y is the time taken to complete a given amount of development at time x. Such presentations of the data are very interesting, and possess advantages as well as disadvantages in comparison with the equations of definite physical meaning, such as that of Arrhenius. For, although they do not seem to give immediately so great an insight into the fundamental processes of the living cell, they are in value independent of advances in physics which may destroy the basis on which the other kind of treatment rests. In other words, their status as short descriptions of the phenomena is safer if not so exalted.

I have now completed what it was necessary to say about the effects of temperature on embryonic growth and development. This knowledge is very valuable, but the unveiling of the limiting factor by temperature control, and the identification of the "master reaction" by fitting growth-curves, are perfect examples of what may be called the "short-cut" method. To a certain type of mind the attractions of the short-cut are too great to be resisted, but, as we have seen, the results are liable to be enigmatic.

2·17. The Effect of Light on Embryonic Growth

The influence of light upon the course of embryonic growth has been little studied, though the results of such work cannot now be looked on with the scepticism of twenty years ago. Schnetzler's paper of 1874 was the first serious contribution to the study, although Edwards fifty years before had stated that frog eggs would not develop at all in the dark, a groundless assertion which had been disproved by Higginbottom and McDonnell. Schnetzler; and Baudrimont & M. de St Ange also found no difference in hatching time, though the former thought that the growth of the tadpoles was more rapid in the light than in the dark. This was substantiated quantitatively by Auerbach and by Yung, who placed freshly laid frog's eggs in vessels containing 60 eggs in 4 litres of water, some in diffuse light, others in the dark. Yung's figures were as follows:

Days development	Size of light tadpoles compared with dark (100)	
	Length	Breadth
25	125	124
30	117	118
60	106	107

It is interesting that the light effects diminish proportionally with increasing age. Lessona and Camerano brought forward field evidence which agreed with the controlled results of Yung, who was later confirmed by Perna and by Chiarugi & Livini. Yung also found that the eggs of *Salmo trutta* hatched 1 day earlier when kept in the light than when kept in the dark, and that the eggs of *Limnaea stagnalis*, the pond snail, hatched in 27 days in the light but 33 days in the dark. Davenport, following earlier speculations of Millet and Blanc, has commented upon the regularity with which developing embryos are hidden away from the light either in the egg or in the uterus, but as this is not the case with animals such as the echinodermata there seems no great significance in the idea. Bodine and Carothers have observed a marked inhibitory effect on embryonic development of certain orthoptera (*Melanoplus differentialis*, *Chortophaga viridifasciata*, *Circotettix verniculatus*) of direct sunlight (not the heat accompanying it) and Miyakawa affirms the same for *Schistosomum japonicum**; but, on the other hand, Page, taking like precautions, reported that shad eggs could be made to hatch 12–15 hours before the controls by allowing direct sunlight to fall upon them. Ruffini's curious observation that the eggs of *Bufo vulgaris* in their jellies always orient themselves with their animal poles pointing toward the light has never been confirmed, but may have significance. According to Goodrich & Scott light has no effect on chick embryo cells growing *in vitro*.

Other researches have been made with the object of identifying the most active wave-length. Béclard very early placed the eggs of *Musca carnaria* behind screens of different colours, and found that the embryos in the green light were the least developed after a definite period, the rest following in the order, yellow, red, blue and violet, in which last light they developed most rapidly. The retarding effect of the green was also observed by Schnetzler. Davidson's results are untrustworthy.

Very striking effects were observed by Yung, whose papers are the most often quoted:

	Length of tadpoles	
Colour of light	1 month	2 months
White	100	100
Violet	117	134
Blue	105	107
Yellow	99	102
Red	83	86
Green	77	All dead

* See also the work of Frederich & Steiner.

As regards echinoderm embryos, we have the following table of Vernon:

	Length °/₀ change from white length	
	Plus	Minus
Semi-darkness ...	2·5	—
Complete darkness ...	—	1·3
Blue	—	4·5
Green	—	4·8
Red	—	6·9
Yellow	—	8·9

Driesch stated that these colours had no effect on *Echinus* embryos. Here the favourable action of violet or blue and the unfavourable action of green are not apparent. Nor in the case of trout fry could Schöndorff find any differences between the colours. The hatching time of various eggs, however, according to Yung, does bring it out again, as Table 75 shows, and the qualitative experiments of Schenk on *Rana* and *Bufo* eggs and Fatigati on other material (infusoria) demonstrate the same relation.

Table 75.

Yung's figures:

	Hatching time in days					
	Violet	Blue	Yellow	Red	Green	White
Loligo vulgaris ...	50	53	58	58	Dead	Dead
Salmo trutta	32	35	34	—	36	35
Limnaea stagnalis ...	17	19	25	36	Dead	27

In 1913 Grein subjected the eggs of *Gadus virens* to light which had passed through the following screens:

		Wave-length allowed to pass
A	Red ...	610–710 μμ
B	Red ...	600–720
C	Red ...	600–700
D	Green ...	440–460
E	Blue ...	All save light green and dark yellow

Hatching times were quicker under *D* than under *A*, *B* or *C*, and quicker under *A*, *B* or *C* than under *E*.

Supino's results, expressed in percentage of embryos hatched after a fixed period, were as follows:

Blue	65	Yellow	43
White	53	Red	39
Darkness ...	45		

It may, on the whole, be concluded that the wave-lengths contained in diffuse daylight which affect embryonic development are the short ones at the violet end of the spectrum. It would be very desirable to go into the whole subject anew in the light of modern technique and modern conceptions.

2·18. The Effect of X-rays and Electricity on Embryonic Growth

Other forms of radiant energy have also been investigated, but the position is very complicated and unsatisfactory. Gilman & Baetjer in 1904, judging from morphological and cytological evidence, concluded that in *Amblystoma*, X-rays at first accelerated development and then retarded it so that eventually the exposed embryos were smaller than the controls.

For the silkworm Hastings, Beckton & Wedd have asserted that X-rays accelerate the developmental time, and (Hastings) that the secondary radiation excited by irradiating copper retards the development of the silkworm. Ancel & Vintemberger in an elaborate and long research, decided that in the case of the chick and the frog, X-radiation had neither an accelerating nor a retarding influence. Nevertheless Colwell, Gladstone & Wakeley believed that they had evidence of retardation by X-rays in the chick, as the following table shows:

Table 76.

Daily dose (P.D.)	Thickness of filter (mm.)	Length of embryo on the eighth day (mm.)
0 (control)	—	22
1	½	4
1	1	4
1	2	4
⅘	½	8
⅘	1	10
⅘	2	12
½	1	13
½	2	13
¼	½	15
¼	1	15
¼	2	15

Their second paper, though mainly morphological, confirmed the earlier one. Richards obtained acceleration working with the egg of the mollusc *Planorbis*, and the opisthobranch *Haminea virescens*. Packard working with *Arbacia*, Lazarus-Barlow & Beckton with *Ascaris*, Bohn with *Strongylocentrotus* eggs, found that short exposures to radium accelerated development and long ones retarded it. Lazarus-Barlow & Beckton found that β and γ radiations alone had the same effect as all three together though the α radiation was 100 times as much as the other two. Försterling obtained retardation of growth in rabbit embryos by irradiating the mother, and other work on mammals was done by Cohn, by Lengfellner, and by Bagg, but the conditions are there so complicated that it is not worth discussing.

The effect of electricity upon embryonic growth has been fairly often tried, but usually in an unintelligent way. Eggs have simply been placed between the electrodes or between the poles of a magnet, and conclusions have been drawn which would probably not bear statistical examination for a moment. Thus Rusconi in 1840 stated that frog's eggs hatched more quickly under the influence of electric currents than otherwise. Lombardini remarked the same effect in amphibian development but noted a large number of abnormalities, as did Fasola. Windle, working with eggs of the trout, found no acceleration of development either under the influence of electric currents or of a large magnet, but he noticed that the hatched trout died very quickly. Rossi found many anomalies in the eggs of urodele amphibia subjected to electric currents, but no other effect. Slater did not find any result at all when he subjected silkworm eggs to a strong magnetic field, nor did Maggiorani when he subjected hen's eggs to one. Finally, Benedicenti found no effect of constant weak currents on the development of echinoderm eggs. The more recent work includes that of Scheminzki on the trout—he could observe no acceleration of development when the eggs were subjected to constant sub-lethal currents, but the membrane at the end of the development was weaker than usual so that the embryos tended to hatch early. Gianferrari & Pugno-Vanoni passed currents of 9000 volts at 450,000 periods/second through suspensions of trout (*Salmo lacustris*) and echinoderm (*Echinus esculentus*) eggs, but the effects observed were merely teratogenic, and the abnormalities produced did not differ, apparently, from those produced in other ways.

The subject still awaits an investigator who will sweep up all this débris into some coherent theory of the action of electrical currents and magnetic fields on embryonic development.

The other factors, such as osmotic pressure, which have been shown to affect growth so greatly in the post-natal stages and in plants, do not exercise so much influence on the foetus, guarded as it usually is from the world around it by the egg-shell or the uterus. Their effects have therefore not been much studied in the case of embryonic growth, and the few data that do exist on the subject will be presented in the section on comparative susceptibility of the embryo at the different stages in its development. The section on biophysical phenomena also contains information on cognate points.

The process of cell-division, as such, is of course influenced by a great variety of factors and substances, as the following table shows:

Table 77.

Substances which accelerate cell-division	Authority
Heat	Laughlin
X-rays	Gilman & Baetjer
Radium emanation	Packard; Bohn; Shumway; and Buddington & Harvey
Thyroiodin	Richards
Adrenalin	Chambers
Alcohol	Calkins; Woodruff
Potassium hydrogen phosphate	Woodruff
Potassium sulphate	,,
Potassium bromide	,,
Oxygen	Godlevski
Sodium and potassium hydroxide	Richards; Loeb
Pilocarpine	Richards
Ammonium hydroxide	,,

In most cases, however, the effects produced by these agents are complicated, and the original papers should be referred to. Naturally, all these substances and factors exercise a depressant action on cell-division if they are applied in too brutal a manner. Moreover, though they tell us a certain amount about the nature of mitosis, they do not much assist in the understanding of the metazoal growth-process, organising mitoses as it does on the large scale. As Richards says, "The advantage gained in the segmentation-stages may later manifest itself in more vigorous larvae than in more rapidly developing ones", so that agents which accelerate cleavage may not accelerate hatching-time. The study of these agents has not led so far to any great advance in our knowledge of the essential nature of growth and development.

2·19. The Effect of Hormones on Embryonic Growth

As regards the effects of endocrine organs upon embryonic growth, practically nothing is known. Willier, who made chorio-allantoic grafts of thyroid in chick eggs, observed that the host embryo was always smaller, and in some cases as much as one-third smaller, than the control. Hanan later, after a thorough study of the difficult question of appropriate dosage, injected 1/600 of a mgm. of thyroxin into the air-space of chick eggs on the 5th day of development. The effect was absolutely nil, as is seen from Figs. 87 & 88 where his points are plotted both for wet and dry weight. There is thus a certain contradiction between the results of Willier and those of Hanan; Okada afterwards repeated Hanan's experiment, and observed no effect on the general appearance, but an 18 % decrease in length and weight of the chicks from the injected eggs. There were characteristic histological changes, mostly in the direction of precocity. Okada's results would fit in with Butler's finding of a decrease in cell-division rate under the influence of thyroxin in *Arbacia* eggs.

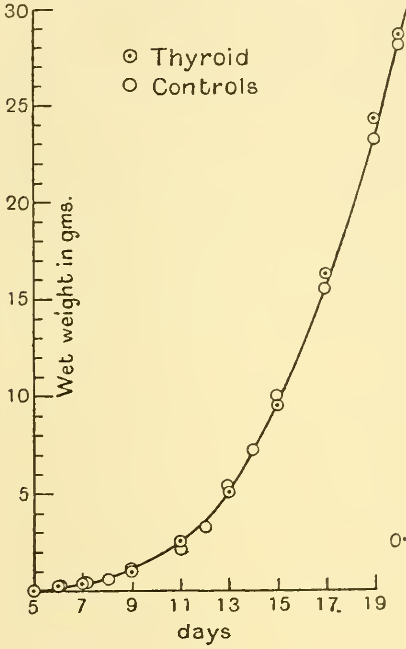


Fig. 87.

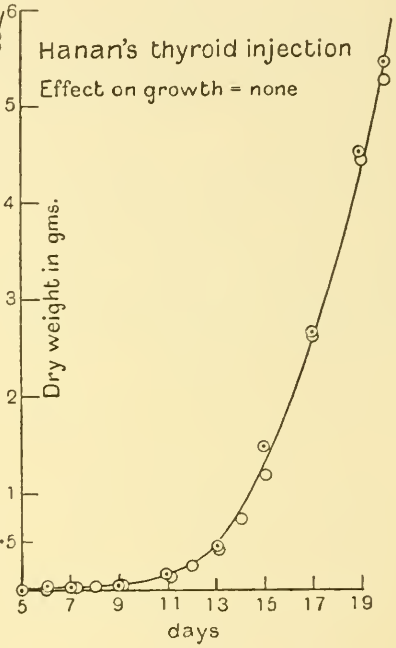


Fig. 88.

Pituitrin has also been injected into hen's eggs, by Cunningham & Stanfield, who obtained the following results:

	Average weights (gm.)	
	Control embryos	Injected embryos
Injection 7th day, opened 9th ...	1·578	1·901
Injection 7th day, allowed to hatch ...	37·4	40·0
Injection 6th day, opened 8th ...	0·950	1·203

This may mean that growth is accelerated, but a larger number of experiments with more complete statistical treatment will be required to settle the question.

A word should be said about the possible total reversibility of growth. Regression to the embryonic state was first brought about by Caullery, and recently Davydov has succeeded in reducing the nemertine *Lineus lacteus* to a state which cannot be distinguished from its blastula. Nothing is known of the chemistry of this process.

In conclusion it may be said that modern embryology has more and more come to follow the lead of Leonardo da Vinci in subjecting all the aspects of the growth of the embryo to exact measurement. In doing so, it has, as we have already seen, put itself in a position to fuse its studies with those of biochemistry and biophysics. The fruit of this union can only be the understanding of the molecular mechanisms underlying embryonic growth and development, from the egg-cell into the newly hatched animal, whose shape and constitution must in the last analysis be regarded as among the properties of what we call matter.

SECTION 3

ON INCREASE IN COMPLEXITY AND ORGANISATION

3·1. The Independence of Growth and Differentiation

The previous section has been concerned solely with the question of increase in size and in mass as the embryo develops; other synchronous processes were perforce neglected. These may be comprehended in one word, differentiation, or increase in complexity and organisation both macroscopic and microscopic. "All generation", said Sir Kenelm Digby, "is made of a fitting, but remote, homogeneall compounded substance; upon which outward Agents, working in the due course of nature, do change it into another substance, quite different from the first, and do make it less homogeneall than the first was. And other circumstances and agents do change this second into a third, that third, into a fourth; and so onwards, by successive mutations that still make every new thing become lesse homogeneall than the former was, according to the nature of heat, mingling more and more different bodies together, untill that substance be produced, which we consider to be the period of all these mutations." Or, as William Harvey puts it, "For though the Head of the Chicken, and the rest of its Trunck, or corporature (being first of a similar constitution) do resemble a Mucus, or a soft glewey substance: out of which afterwards all the parts are framed in their order; yet by the same operation, and the same Operatour, they are together *made* and *augmented*: and as the substance resembling glew doth grow, so are the parts distinguished. They are Generated, Altered, and Formed at once". Having discussed then in the preceding section the "augmentation" of the embryo, we have now to discuss its "making" or in Harvey's other term, its "framing".

Framing is undoubtedly not an increase in complexity alone. Woodger has well said, "It is often stated that organisms are just complicated physico-chemical reactions, and it is because they are so complicated that biology has so far made so little progress. But it is evidently not simply a question of complicatedness, because there are plenty of complicated goings on in the world which no one would mistake for organisms. The 'something going on' which we call a thunderstorm, for example, is very complicated. An organism from some points of view is comparatively simple, otherwise biology would not have got as far as it has, and this simplicity appears to be the outcome of its organisation. What happens in development is not merely an increase in complexity nor an increase in spatial structure, but a gradual rise in the level of organisation of the developing organism".

It has often been said that the interesting thing about any magnitude is not so much its absolute as its relative size, and not so much its relative size as the rate at which its relative size is changing. The first question to ask therefore in the discussion of the physico-chemical aspect of differentiation is whether the rate of differentiation is the same as the rate of growth at each embryonic stage. That the two processes are independent in the sense that one can be induced without the other has long been known. Panum, for instance, as long ago as 1860 observed chick embryos with well-developed blastoderms but no primitive streak, but he did not associate this with any definite causative factor. Then Broca found he could get this growth without differentiation by keeping fertile eggs for a month or more at room temperature before incubating them, and Dareste observed that very high as well as very low temperatures maintained during the first 24 hours of development would bring about the same effect. Rabaud confirmed this: for further information see the review of Tur. Finally, Edwards found that cell-division without embryonic organisation always occurred in eggs incubated from 20 to 27°. Continued growth may take place also in the primitive streak stage as well as in the simple blastoderm when there is not a sufficient degree of temperature to permit of normal differentiation. Fig. 89, taken from Edwards' paper, shows the effects he obtained. The controlled action of temperature can also bring about nuclear division without corresponding cell-division in *Echinus*, according to

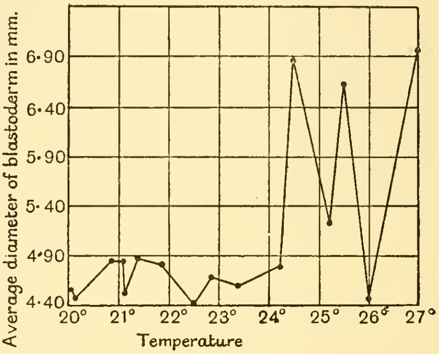


Fig. 89.

Driesch, and many other workers have observed these polynuclear undifferentiated masses in other embryos with abnormal temperatures. Even in bacteria growth in size can take place without cleavage, according to Henrici, and the work on the endocrine control of amphibian metamorphosis affords other instances of continuous growth without differentiation.

The most remarkable case of this, perhaps, is contained in the work of Hoadley, who transplanted embryonic organs of the chick on to the chorio-allantoic membrane and allowed them to develop there until they had reached a degree of differentiation equivalent to that of the controls. Then by making and weighing wax models he ascertained the relative weights, and always found that the controls were much heavier. But there was a direct relation with age, for the younger the transplant at the time of transplantation the smaller the eventual (fully-differentiated) organ, thus:

	At the time of transplantation		Control : Transplant = 1 : x
Organ	Embryo age (hours)	No. of somites	x (after about 8 days)
Spinal cord	48	28	0·353
Eye	48	28	0·219
,,	35	14	0·098
,,	20	0	0·0136
,,	4	0	0·00075

Another instance of the independence of growth-rate and differentiation-rate is afforded by the genetic races of rabbits which differ considerably in size. At birth the large-sized race is twice as heavy as the small-sized race, and at the adult stage, three times as heavy. In 1928 Painter observed that equally differentiated 12-day embryos showed the characteristic weight-differences and thought that the effect might be of endocrine origin, but Castle & Gregory were later able to trace it back as far as the morula stage. The large-sized race showed consistently more rapid cell-multiplication and size increase in the embryonic period with unaltered differentiation-rate. There is no difference in egg-size: but the large-sized race grows more quickly, thus:

	Embryo age in hours	Average no. of blastomeres	Diameter of blastodermic vesicle
Large-sized race	48	21·75	—
Small-sized race	48	14·00	—
Large-sized race	100	—	31·4
Small-sized race	100	—	16·3

Another good instance of this dissociation of the fundamental embryogenic processes is seen in the interesting work of Twitty, who studied the development and the nature of the action of the cilia on the skin of amphibian embryos, previously described by Assheton and Woerdemann. He found that the polarity of the ciliary cells is determined during the closure of the neural folds, for cilia grafts rotated 180° before that stage beat in the same direction as the cilia of the adjacent ectoderm, but cilia grafts transplanted later retained their original direction of waving. Thus the determination here (in *Amblystoma punctatum*) occurred much later than the main point of chemodifferentiation (see p. 575). Now in embryos allowed to develop at low temperatures, Twitty found that this ciliary polarity appeared at a much earlier stage. Evidently the determinative process had been thrown out of gear with the morphological ones by the cold, and the two were proceeding more or less independently. Again, certain treatments, as is well known, inhibit segmentation, and cause the production of cilia, while histogenesis and organogenesis are easily separable as is seen in teratological experiments (Ranzi) and in explantation work (Hoadley)*.

3·2. Differentiation-rate

Under certain conditions then, such fundamental processes as growth and differentiation can be shown to be proceeding independently of each other. That their normal velocities differ might or might not be the case. All who have had any practical contact with embryology know that there is much more difference in shape and form between a chick embryo of the 2nd day and one of the 5th than there is between one of the 12th day and one of the 15th. But the difficulty has always been to evolve some method of measuring change in shape—a more elusive entity than change in weight. Murray, however, has made an admirable attempt in this direction, choosing as the organ for investigation the heart, which, as we have already seen in the work of Schmalhausen, appears to keep pace exactly as regards growth with the embryo as a whole. He tested this by measurements of the surface area of the organ, calculating the percentage growth-rate of the heart from Cohn's work with a projectoscope and a planimeter. The result was one of the usual descending curves which closely resembled that for the embryo as a whole, and the log. surface area of the heart/log. age of embryo relation was also a straight line.

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* Thus Waddington, working with chick embryos cultivated *in vitro*, found that the 12-somite stage was much smaller than the corresponding stage in the egg.

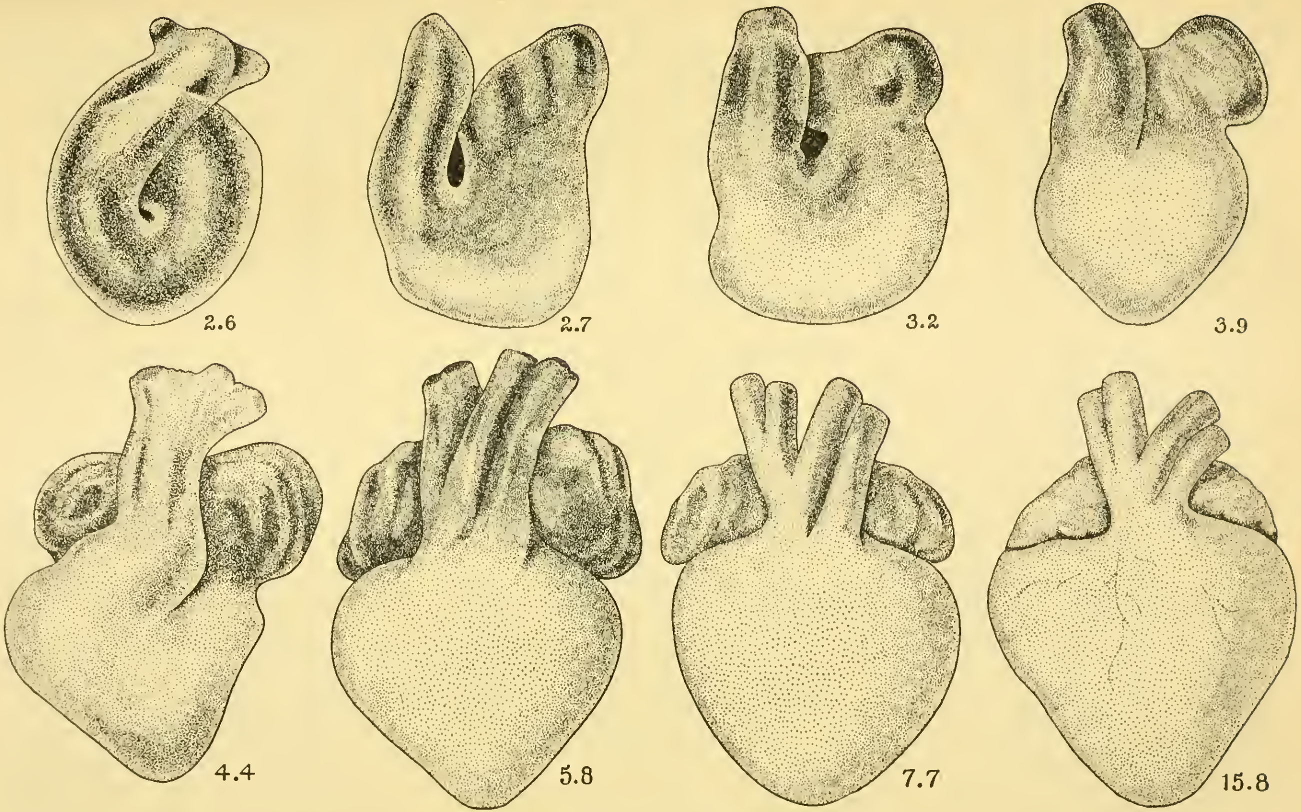
2.6
2.7
3.2
3.9
4.4
5.8
7.7
15.8

Fig. 90. The numbers give embryo age in days.

"No satisfactory method", Murray said, "of measuring changes in form quantitatively is known, so that it was necessary to resort to the expedient of selecting forms spaced by a visual impression so as to represent approximately equal degrees of gross change. In other words, from a series of drawings made at frequent intervals, certain ones were chosen which seemed by inspection to be equally spaced from one another in respect to their relative complexity of form. The test is thus necessarily arbitrary and open to criticism because of its subjective nature. By taking the average result of many eggs it was then determined what were the exact incubation ages of the embryos with heart forms such as those selected." The illustration shows these clearly (Fig. 90). The reciprocals of the time intervals between successive drawings were used as rough criteria of the rate of form development. The graph showing this done is reproduced in Fig. 91, from which it is clear that the rate of evolution of external form falls precipitously at first, and then ever more slowly, essentially resembling in this way the instantaneous percentage growth-rate, according to Schmalhausen, or the averages of the steps in the fall of the instantaneous percentage growth-rate, according to Brody. Growth

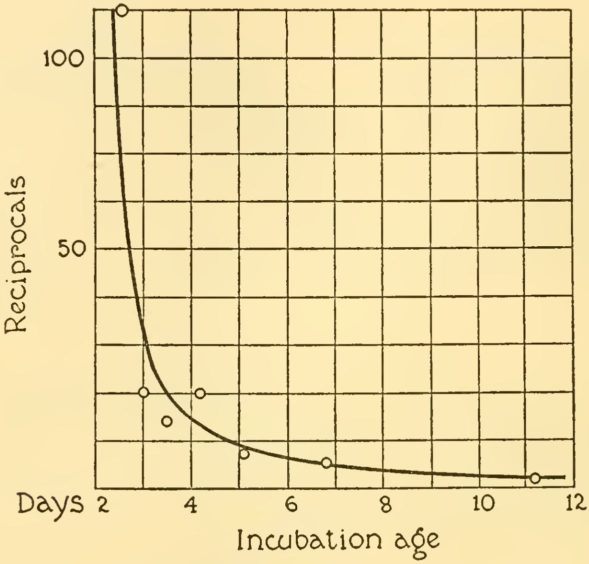

Fig. 91.

and form would therefore seem to be what Murray calls "aligned" with one another, for the most marked form changes occur at the initiation of embryonic development when the growth-rate is at its highest. Murray's interim method affords a basis of quantitative comparison between growth-rate and differentiation-rate. But if these two processes change together with like velocity, others do not. The shifts of chemical and physical change within the embryonic body follow an entirely different course In 1925 I had drawn attention to the fact that, making allowance for the increase in size of the embryo and for its consequently increased daily turnover of matter and energy, chemical activity seemed to be more intense at the end of development than at the beginning. "One pictures", I had said, "the gradual elaboration of structure up to the beginning of the third week, followed then by a burst of chemical activity consequent upon the assumption of function by the organs already formed. In this hypothesis is involved the view that in the first fortnight chemical change is limited to those compounds which are required mainly for structural purposes, while toward the end of incubation the opening up of functional operations causes marked and profound chemical changes of other kinds." This was proposed in connection with the three periods which modern embryologists have come to recognise in all embryonic development. The same idea was elaborated further by Murray in relation to change in growth-rate and differentiation-rate. Taking the rate of change in chemical constitution, he concluded (largely from his own experiments) that the most marked changes occurred after the 10th day, not before it, as is the case with growth-rate. "Internal integration", he said, "may be regarded as a process characterised by the concentration of solid substances within the body, whereas chemical differentiation is a change in the composition of the solid substances thus integrated." Fig. 92, taken from Murray, demonstrates these relationships, for it may be observed that the percentage of ash in the embryo, the percentage of total solids and the percentage of fat, together with the rate of production of carbon dioxide, all rise or fall in the same way, i.e. more rapidly at the end of development than at the beginning, and precisely opposite to the growth-rate and the differentiation-rate. Thus, just as morphological growth and differentiation take place more rapidly the younger the embryo, so chemical growth and differentiation take place more rapidly the older the embryo. A note of caution must be inserted here, however, in view of the fact that, until we know more about the behaviour of individual organs in the light of these conceptions, we cannot estimate the part played by ossification, for instance, in producing these overall results. Murray also afterwards found that the curve relating age with oxygen absorbed by the embryo per gm. per day did not decline quite like the carbon dioxide evolution curve, i.e. slowly at first, and then quicker and quicker, but was rather **S**-shaped, the slowest rate occurring at the 10th day. His line for this, however, depended for the initial fall exclusively upon one very high point for the 6th day, and this may quite possibly have been wrong, in which case the curve for oxygen consumption would fall with the curve for carbon dioxide output.

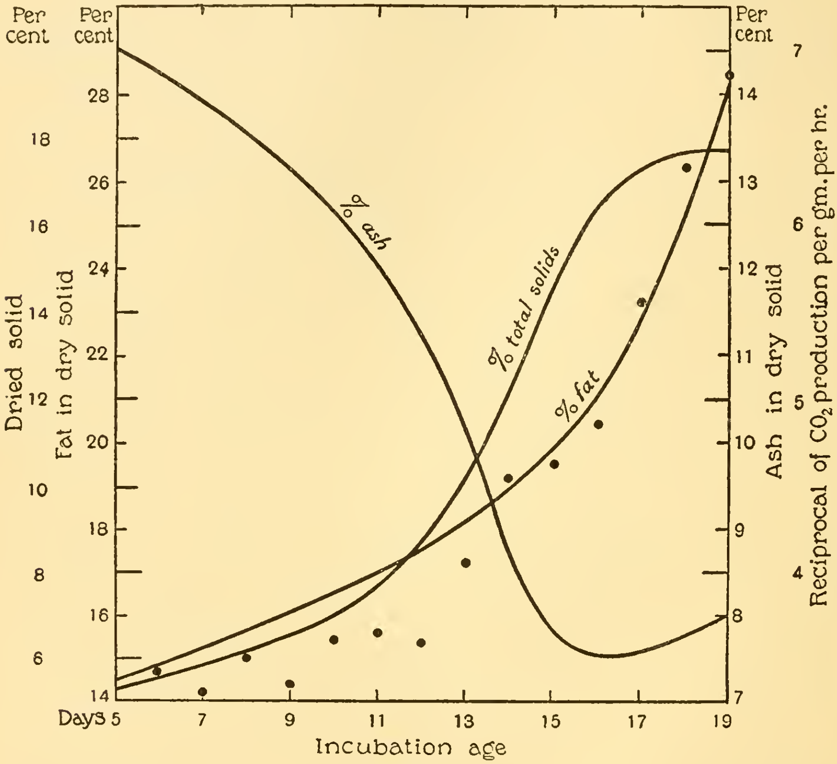

Fig. 92.

Murray next showed that the curves for growth-rate of heart tissue in explanted culture, obtained by himself working with Cohn & Rosenthal (see p. 461), fell in the same way as those for the intact embryo in its egg, but that the curve for latent period (see p. 462) behaved, on the contrary, exactly like those for integration and chemical differentiation, rising very slowly at first, and thereafter more rapidly. Thus chemical constitution, metabolic rate, and latent period of explant growth are to be correlated and distinguished from growth-rate both in the intact animal and in tissue culture. Fig. 93 shows the difference between growth-rate and metabolic rate, as judged from the rate of carbon dioxide production.

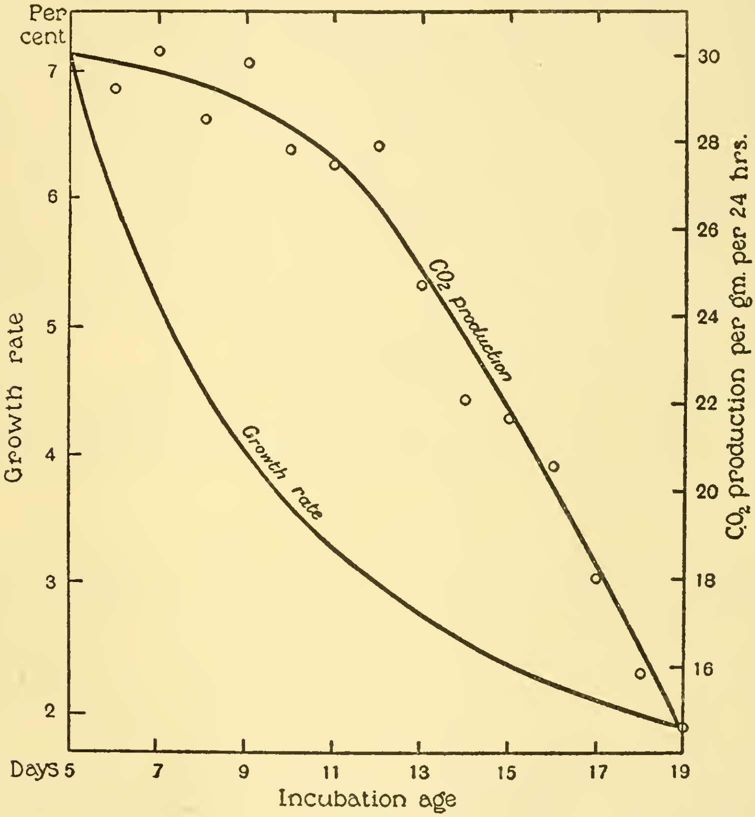

Fig. 93.

As a result of this analysis of embryonic development, at least five fundamental processes may be distinguished, falling into two groups. The first group consists of those processes which have their maximum rate of change early in development, and it includes both growth-rate and differentiation-rate. The second group consists of those processes which have their maximum rate of change late in development, and it includes, firstly, the concentration of solids in the embryo (called by Murray "chemical growth"), secondly, the increase in complexity or change of composition in the solid substance (called by Murray "chemical differentiation") *, and thirdly, the metabolic rate or respiratory intensity. These two groups of processes correspond to two type curves which in some circumstances show skew symmetry round a central point. The first group has been termed by Murray the group of "primary integration" and the second one the group of "secondary integration", and he has in various papers associated the two groups with the primary and secondary redistributions of the evolutionary process in Herbert Spencer's scheme, or, as they are often called, simple and compound evolution.

Murray found that the surface volume ratio was also a member of the first group, changing most rapidly in the earliest stages and falling in a way not unlike the growth-rate. He calculated it from the formula of Meeh:

$$S = KW^{\frac{2}{3}},$$

and, although such an application to the chick embryo rests inevitably on several unproved assumptions, it is probable that his curve is not wholly misleading (see Fig. 94). More interesting still, he found in a later paper that the absorption curve (the grams absorbed per gram dry weight per day) was not a member of either group, for it was sigmoid, falling rapidly for a time, then less rapidly, and then more rapidly again. His absorption curve, however, was derived from the oxygen measurements which have already been criticised, a fact which might account for its S-shaped character. About the same time, I also calculated the curve of absorption-rate, not from respiration experiments but from direct measurements of protein, fat and carbohydrate, and I obtained a curve belonging to group 1, i.e. changing most rapidly at the beginning of development. It is very interesting that the curve of absorption-rate should be found to follow the curve for surface/volume ratio (see Fig. 253).

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* This process of "chemical differentiation" must not be confused with "chemo-differentiation" (see p. 571).

Murray's presentation of the facts does bring an order into a realm where order is much needed. But it is not altogether easy to see why increase in dry weight should be regarded as "chemical growth", for water is just as much a chemical compound as anything else. Why should the title of chemical compound be restricted to those bodies which do not happen to be liquids at room temperature? And the wetness of the embryonic body must also exercise a profound influence on the speed and nature of the chemical reactions going on within it, as is emphasised in the work of Ružička and his school. There is therefore some reason for objecting to the term "chemical growth", though the simple fact that rate of increase in dry weight follows an opposite course from rate of increase in wet weight (i.e. "growth") is plainly of importance. There is less reason for objecting to the term "chemical differentiation", for Murray uses it to refer to such entities as percentage of ash and percentage of fat, and shows that, although for by far the greater part of the embryonic period the

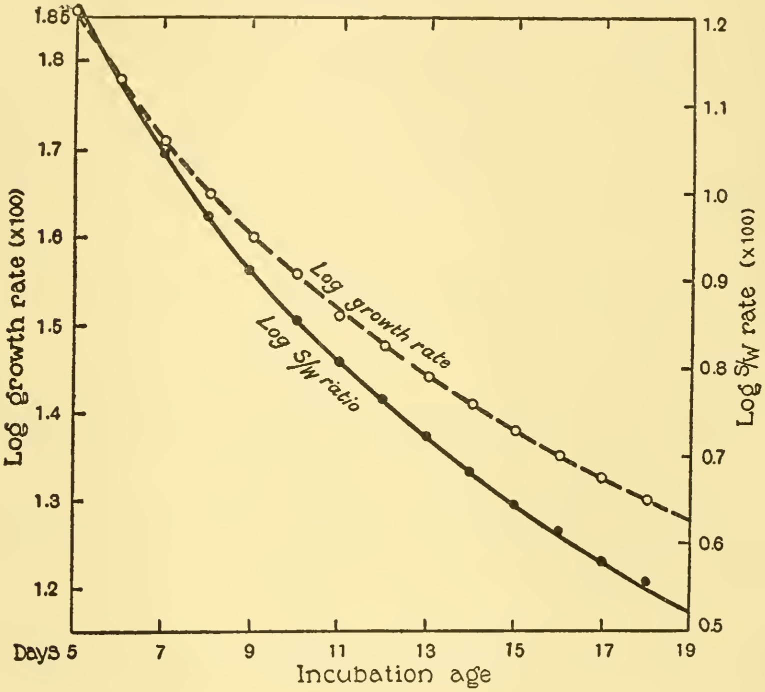


Fig. 94.

former is descending and the latter ascending, they are doing it in the same phase, i.e. slowly at first and more quickly afterwards. Probably the most significant result which emerges from Murray's symmetrically diphasic plan is that growth-rate and differentiation-rate go together, and in opposition to metabolic rate.

Enriquez's paper of 1909 contains a foreshadowing of the idea of chemical differentiation (see also Scammon & Ness).

3·3. Chemical Processes and Organic Form

In considering the processes of differentiation in the embryo, there has been much disinclination to admit their physico-chemical nature. It is the great credit of His that he took the lead in this direction, pointing out that processes such as the production of the neural and amniotic folds were the inevitable results of unequal growth controlled by physico-chemical factors taking place in what was, to start with, an undifferentiated sheet of embryonic cells. His's artificial blastoderms, again, made of pills of dough to which varying amounts of yeast in various places are added, provide a close model for the embryo. D'Arcy Thompson has described the incredulity and opposition which the views of His met with, especially after the publication of his classical letter in the *Proceedings of the Royal Society of Edinburgh* in 1888. As Garbovski put it, "it is absurd to treat the living being as if it were made up of vesicles, cylinders, and plates, and not of vital units". Embryologists such as Hertwig and Balfour held that, in the study of development, a sufficient causal explanation of one stage had been given when the immediately preceding stage had been adequately described. "My own attempts", said His in a famous passage, "to introduce some elementary mechanical or physiological conceptions into embryology have not been generally agreed to by morphologists. To one it seemed ridiculous to speak of the elasticity of the germinal layers; another thought that by such considerations we put the cart before the horse; and one more recent author states that we have better things to do in embryology than to discuss tensions of germinal layers and similar questions, since all embryological explanations must necessarily be of a phylogenetic nature. This opposition to the application of the fundamental principles of science to embryological questions would hardly be intelligible if it had not a dogmatic background. No other explanation of living forms is allowed than heredity and any which is founded on another must be rejected—yet to think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism."

Such opposition to mechanical explanations in embryology has of course long been dead, and there are many authors who have put forward such theories to account for the phenomena of differentiation in the embryo. But these theories are for the most part quite physical, depending on physical properties such as elasticity and torsion, so that they cannot be considered in detail in this book. It is also very unfortunate that, owing to the exceedingly small size of the parts undergoing such changes, practically no direct work has been done, and investigators have focused their energies on the preparation of models, made of a variety of materials, such as rubber and plasticine, which can be made, like the yeast pills of His, to exhibit the phenomena of gastrulation and the like. Roux in his valuable review of the technique of "Entwicklungsmechanik" has described such rubber models, and an even greater collection of them is to be found in the paper of Rhumbler. Rhumbler gives in full the literature on this subject (e.g. Morgan; Schaper & Cohen; and Spek), and has many illustrations of invagination models, etc. (see especially his Section F onwards). These investigations are interesting indeed, but they do not contribute a great deal to our knowledge of what happens in the early stages of embryonic development, although they certainly set forth a number of ways in which it might conceivably happen. Robertson's theory of cell-division, again, in which lecithin was supposed to be broken down at the two nuclear regions of the dividing cell to provide phosphoric acid for new nuclein, and the resulting free choline to diffuse away, reaching its greatest concentration in the equatorial plane at right angles to the line joining the two nuclei, was never shown to hold in actual fact. Like so many of the "Nachahmung" models, it rested upon an unwarranted simplification of the material under discussion. Nevertheless, it was an ingenious suggestion, and, in spite of McClendon's criticisms, it may still be found to contain a modicum of truth, but the reason why it and others like it will not be taken up in detail here is because they are not sufficiently close to the facts. The purpose of this book is to give all the facts that are known about the physico-chemical aspects of embryonic development, and not the theories, which, indeed, would demand a much larger treatise.

That there is at present a certain gulf between physico-chemical research and the "form" and "shape" of the morphologists is a fact which must be faced. In present-day biology, there lives on, still very hale and hearty, the essence of the distinction made by Aristotle between ὕλη and εἶδος, matter and form. It is probably at bottom this which inspires the statements so often made by morphologists that do what one will with chemical methods, the meaning of form in animals will always elude one's grasp, for it belongs to another order of existences, a range of concepts intrinsically remote from physics. In so far as the form of living organisms is an expression of a degree of organisation higher than anything with which the sciences of the non-living world have to deal, it is true that we have to deal with something very different from mere heaps of molecules, but crystal form and the colloidal state, which exhibit an intermediate degree of organisation, exist in the inorganic world and can be dealt with by the quantitative methods of physics and chemistry.

Examples of the extreme morphological point of view are common; thus Cunningham in 1928, discussing chemical embryology, remarked of two eggs in the same incubator, "Why are the bones formed in one case the bones of a chick and in the other case the bones of a duckling?" For him the fundamental problem is, not how does the rabbit get out of the hat, but why a particular kind of hat should produce a particular kind of rabbit. In one sense the question is simply a special case of the general question, why is the universe what it is and not something else? and thus reduces to a query concerning the fundamentally alogical character of the universe. With such conundrums the scientific investigator is not concerned and Cunningham should have addressed his inquiry to metaphysicians. Cunningham's question had already been raised by C. D. Broad. "The ultimate question", said he, "is, how do these particular material systems called organisms come to have their particular structure or components. So long as we explain their origin by laws, whether mechanical or otherwise, we are merely referred back to earlier collocations of matter, and so on *ad infinitum.* The explanation in terms of a designing mind on the analogy of humanly constructed machines seems to involve a circle or to end in a mind so different from any that we know that the analogy fails and it is hardly worth while calling it a mind. The explanation by entelechies rests on a confusion and avoids no difficulty which is raised by the notion of an external designer. The problem, as far as I can see, is extra-scientific and quite insoluble."

There is a sense, however, in which Cunningham's question has a meaning for science, and all that can be said in answer to it is that the exact biologist has a hope, a belief, that in the long run the outward forms and shapes of living animals are as much dependent on the properties of what we call matter, as anything else about them. To object that this is to endow atoms and electrons with occult properties and potentialities is not reasonable, for the only alternative is to abandon all hope of bringing form and shape within the coherent scheme of the scientific world-picture. We cannot resign ourselves to leaving them out in this way, and there is the less cause for despondency when we see what great progress has been made along such lines of research as that pursued by d'Arcy Thompson and summarised in his *Growth and Form*.

Nevertheless there are real difficulties in this subject, and J. H. Woodger, who has acutely felt them, has even gone so far as to maintain that biology may for ever consist of two irreconcilable divisions, morphology and physiology. Experimental and causal embryology, in his view, is only physiology disguised. He would regard morphological description as an end in itself, and the ultimate aim of the morphologist to come down to solid geometry instead of to causal relationships. There may be in this view an element of truth, but in so far as any morphologist holds such an opinion of his goal he must admit himself to be an artist searching for the aesthetic experience of significant form rather than a scientific investigator seeking for understanding of how the thing works—surely the essence of scientific explanation.

Another thinker who has vigorously opposed the extension of physico-chemical concepts to include morphology is E. Rignano. In a discussion of the relations of biochemistry with embryology he said, "Now what do the ultra-mechanists do in the presence of these teleological manifestations of the generative and regenerative processes? They direct all their efforts to an attempt to prove that given chemical substances exercise a morphogenetic action on particular developments, hoping to conclude triumphantly that the entire series of morphogenetic phenomena, constituting the ontogenetic development, may be explained completely by physico-chemical action. But in this attempt they have mistaken a mere release of morphogenetic activities already potentially in the developing embryo for a genuine morphogenetic action". Now it is easy to adduce in the light of biochemical researches, chains of causes which can have the effects seen in the developing embryo. One could start with the experiments of Huxley on the morphogenetic action of thyroxin. We know from Ahlgren's work that thyroxin has definite effects on oxidation-reduction mechanisms *in vitro*, just as other hormones have, and anything that may locally affect oxidation-reduction mechanisms has every chance of affecting the local fatty acid concentration, as is indicated by the work of Hopkins, so that in due course the lipocytic constant or some other such cellular value (cf. Mayer & Schaeffer) will alter and change correspondingly the surfaces of the intracellular phases in that region, with the final outcome that, as in the models of Warburg, one chemical substance may be formed instead of another. These two alternatives may be thought of, for instance, as scleroprotein on the one hand or phosphoprotein on the other, and thence it requires little imagination to picture the most profound morphological changes taking place. These causal chains are being unravelled every day.

But Rignano was probably prepared to admit the cogency of these mechanisms within their own sphere; what he wanted to know was, why should one embryo pass through all these changes and come out at the end a dogfish, and another pass through them and come out a skate. The standard answer of exact biology has, of course, been that the genetic constitution of the former animal governs the chemical morphogenetic processes, catalysing this and inhibiting that, so as to produce the results we find. Rignano, Haldane, and their associates have often replied that such a preformationism implies a complexity too great to be imagined when it is faced with the facts of biology as a whole, but this depends on one's imagination. The alternatives, it might be argued, are much worse.

The adversaries of genetics too often seem to suppose that every one of the infinite number of characters which they see in a given animal has to be *represented* in some way, within the nucleus. They forget that large blocks, as it were, of the specific characters may be the result of single processes set in action by a gene, and do not consider the possibility that a good deal of morphogenesis may be associated with a "delegation of function", the gene activating secondary key-factors, just as statesmen delegate many of their duties to subsidiary but competent officials. This possibility becomes a probability when the apparently mysterious and arbitrary grouping of characters is considered, colour *x* always going together with pollen-shape *y* and so on.

In any case there is no need to load all the responsibility for the adult skate and dogfish on to their genetic equipment; any more than we need suppose the constitution of their eggs to differ simply by the presence of two different entelechies, as Driesch would say (see p. 22). It is true that in many respects the chemical constitution of eggs is alike in different animals, but only when very broadly considered. We know that the provision of amino-acids is by no means the same, and serological differences, which have so far only been touched on by a few workers (see Section 19), are likely to be of much importance. Riddle has also emphasised the importance of the environmental factors in contributing to the final result of ontogenesis, so the three principal sets of morphogenetic causes which exact biologists at present accept are thus:

(i) The genetic constitution of the egg-cell.
(ii) The physico-chemical constitution of the egg's raw materials.
(iii) The environment during ontogeny.

Woodger observes that modern genetics owes a debt to the preformation theory of the eighteenth century. The old theory identified the "immanent factors" in the egg with the whole of the newly separated individual, and imagined nothing but an increase in volume. The modern theory identifies the immanent factors with certain small bits of the individual, such bits being thought of as related to the qualities of the individual as cause to effect. Woodger considers that we shall get on better by sharply distinguishing between genetics and embryology instead of by attempting to fuse them, as is now the general aim, for he regards the formation of *parts* as fundamentally or causally separate from the determination of *characters*. I must refer those who are interested, to the original discussion, but if we are not to treat parts as characters, an entirely new conception of evolution will be required.

The difficulty of fusing morphology and physico-chemical biology is, in fact, very real, and Rignano's objections, though they are far from insuperable, are not merely restatements of finite teleology. They bring up the question of how any real epigenesis can take place at all, i.e. how from moment to moment the level of actual organisation in the embryo can rise, a question of much theoretical importance to which I shall return in the Epilegomena. But chemical embryology will never allow itself to be restricted to the description of relatively superficial events in the life of the embryo, such as the appearance of enzymes in the digestive tract. It will insist on expanding physics and chemistry, if necessary, to cover the animal level of organisation. It will affirm that if we knew all that there is to be known about the physico-chemical constitution of the egg, we should be able to predict the results of its development. This affirmation does not imply that we should be able to make such predictions before any given case had been observed; for the fact of emergence is real and it is true that knowing all the properties of simpler systems, including how they could combine with one another, does not tell us how in point of fact they actually do combine. The schemes of science are resultant, not emergent, they cannot describe the complex systems before they have been observed, but they can and do offer reasonable causal explanations of them with reference to their simpler factors, after they have been observed. In this way we are not sure that physico-chemical embryology will ever be able to say what a hitherto unknown egg will develop into, but we do expect it some day to be in a position to offer a reasonable causal explanation of the origin of all measurable properties of adult living beings from the measurable properties of their eggs. And form is evidently one of these, just as is physical constitution.

There is a great deal of confusion at the present time about such questions and very few workers stop to ask themselves what is the true aim of their studies in causal or exact biology. Moreover, many biologists are uncertain as to the meaning of the facts with which this book is dealing. "I have not so far been able to discern", writes a Belgian embryologist to me, "the truly explicative value of quantitative measures in embryology. Far be it from me to minimise the interest of the analysis of metabolism in development, but I have the impression that it is more thrilling for the biochemist than for the embryologist. For the latter the cardinal problems remain the setting-in-action of development, differentiation, or heredity, and their mystery lies wholly in the laws of cell-life. Now these laws have not so far been elucidated, and we hardly know anything of how one part of the cell acts on another; thus there remains an enormous qualitative task to accomplish before we can begin to enter on a numerical phase. I have been thinking of all this in reading the monograph of M. Rapkine on the energetics of development, where the physico-chemical facts are presented in a plain sort of manner lending itself well to meditation. But I confess that it all remains sibylline, enigmatic, to me, and some acute friends have admitted the same embarrassment. Where is the explicative value, the light, the link between all these facts so interesting, yet so isolated? I cannot hit on it." This excellent passage, which my friend has kindly allowed me to quote, expresses admirably the doubtful air with which many biologists regard the extension of physico-chemical concepts into morphology. In answer I pointed out that chemical embryology is a very young science and if more attention had been devoted to it in the past, would not show the blank spaces and the gaps of which my friend complained. In a word, Roux's decision to concentrate solely on the revelation of the "secondary components" in embryogenesis has had momentous effects, but there were always those who could not resist the temptation of going deeper down to the "primary components" before all the mass of facts had been dealt with on the more superficial level. Among such miners at the deeper levels are the physico-chemical embryologists. It is true that an enormous qualitative task remains to be accomplished, but there is room enough for both kinds of work and it will surely be through the close co-operation of both kinds of worker that the facts will lose their sibylline and enigmatic character.

3·4. The Types of Morphogenetic Action

In order to have some concrete idea as to how the processes of morphogenesis may be supposed to go on, and to form a background for chemical facts, one may construct a table in which are placed all the different kinds of process that may go on during differentiation. Such classifications have been made by Kellicott and by Jenkinson, and tables constructed from them are given here. (Charts IV and V.)

Jenkinson's table may be taken first. The movements of single cells which he enumerates are of much importance. The lower-layer cells of the blastoderm of elasmobranch fishes, for instance, are free and

CHART IV

Jenkinson's table:

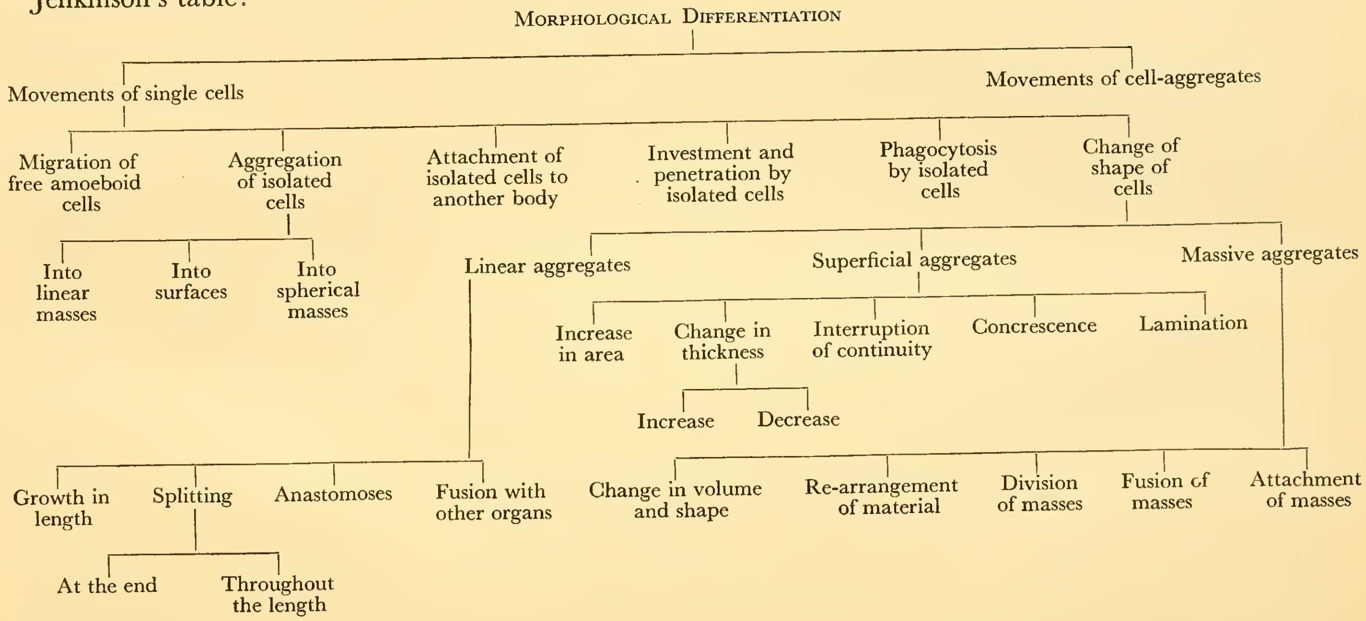
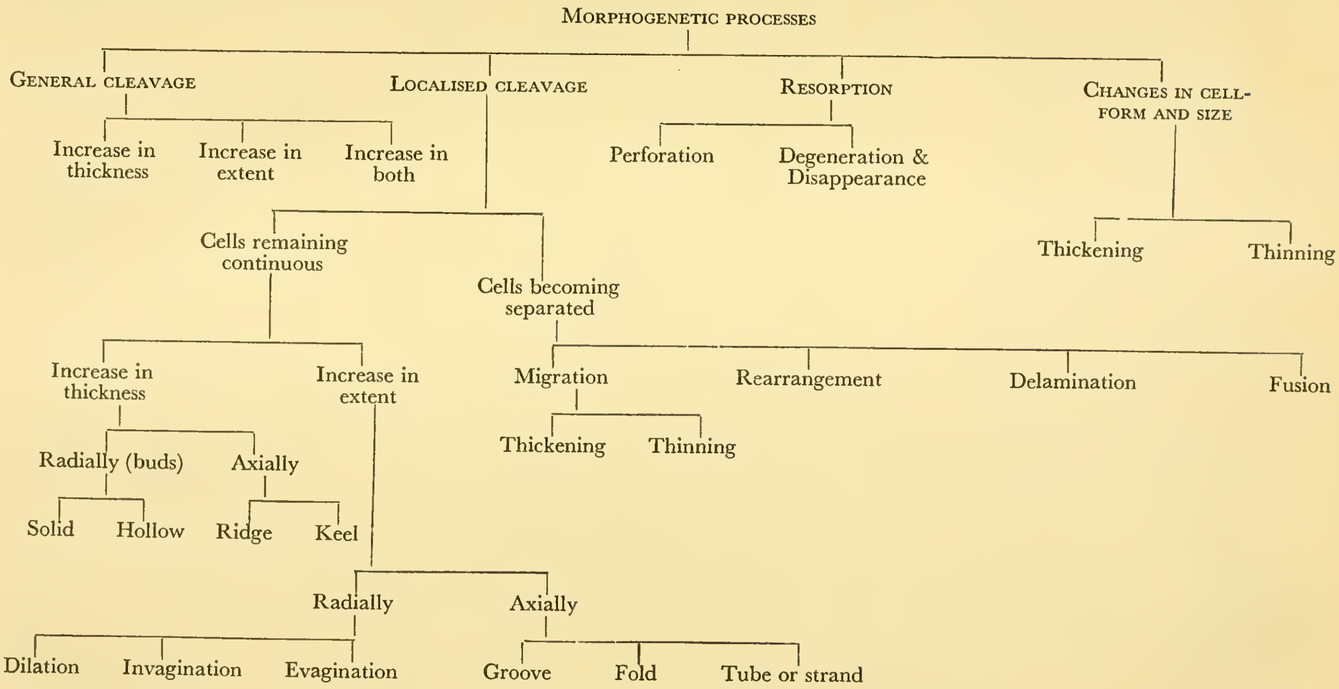

Morphological Differentiation
Movements of single cells
Movements of cell-aggregates
Migration of free amoeboid cells
Aggregation of isolated cells
Attachment of isolated cells to another body
Investment and penetration by isolated cells
Phagocytosis by isolated cells
Change of shape of cells
Into linear masses
Into surfaces
Into spherical masses
Linear aggregates
Superficial aggregates
Massive aggregates
Increase in area
Change in thickness
Interruption of continuity
Concrescence
Lamination
Increase
Decrease
Growth in length
Splitting
Anastomoses
Fusion with other organs
Change in volume and shape
Re-arrangement of material
Division of masses
Fusion of masses
Attachment of masses
At the end
Throughout the length

CHART V

Kellicott's table:


Morphogenetic processes
General cleavage
Localised cleavage
Resorption
Changes in cell-form and size
Increase in thickness
Increase in extent
Increase in both
Perforation
Degeneration & Disappearance
Thickening
Thinning
Cells remaining continuous
Cells becoming separated
Increase in thickness
Increase in extent
Migration
Rearrangement
Delamination
Fusion
Thickening
Thinning
Radially (buds)
Axially
Solid
Hollow
Ridge
Keel
Radially
Axially
Dilation
Invagination
Evagination
Groove
Fold
Tube or strand

have amoeboid properties; they form an example of the first class. Linear aggregation would be found in the formation of blood-vessels and superficial aggregation in the formation of the yolk-sac in certain mammals. Aggregation of cells into spherical masses is common in organogeny, e.g. the origin of the spleen. The attachment of isolated cells to another body takes place when tendon is united to bone, and penetration by isolated cells into another body when the vitreous humour of the eye is secreted. Phagocytosis plays a large part mostly in post-embryonic differentiation such as the absorption of the tadpole tail, and perhaps in insect metamorphosis, but change of shape in cells is usual in all embryos, i.e. from flat to columnar or from neuron to neuron plus axon.

The movements of linear masses are more important still; thus growth in length is seen in the back growth of the segmental duct during the formation of the somites. Splitting occurs at the end of linear masses whenever they branch as nerves and vessels frequently do, and throughout their length in, for instance, the elasmobranch segmental duct. Anastomosis is very common within the circulatory system, and fusion with other organs occurs in the case of the vasa efferentia and the mesonephros, as also in the case of the formation of the pituitary body. The movements of superficial aggregates, sheets or layers, involve various alterations, such as increase in area in the growth of the chick blastoderm over the yolk or the formation of the mammalian blastocyst. This increase in area may be uniform all over the surface, in which case the structure at the end of the process will have much the same form as it had at the beginning, or it may not be, in which case invaginations and foldings will be produced. The optic cup is produced in this way, as also is the medullary groove. As will later be seen, this unequal expansion of surface has important connections with the theory of metabolic rate. The sheet of cells may also thicken as the mammalian trophoblast does in the formation of the placenta, or it may become thinner as the roof of the thalamencephalon and medulla does, or the outer wall of the lens. If it goes still further in this direction, it may break altogether and a perforation be formed. This is what happens to the floor of the archenteron in the amniota, and in many other instances. Again, two separate layers or sheets of cells may fuse into one by a process of concrescence, and this is what happens when the medullary folds close over the medullary groove uniting flatly together, or when the stomodaeum unites with the gut. Here the concrescence is one of surface, but it may also take place between the margins of the layers, as in the union of the embryonic plate with the trophoblast in some mammals. Finally, a layer may split into two by lamination, which is what happens in the inner wall of the pineal vesicle in lizards.

Aggregates of cells in mass formation may also be subject to quick and profound changes of shape. The outgrowth of the limb-buds affords an example of simple increase of size and weight, but more complicated processes may take place also, such as the elaboration of internal cavities or special internal structures. The blastocoele cavity offers itself as an obvious instance of the former alteration, but another one would be the origin of blood-vessel cavities from solid ropes of cells. Internal re-arrangement is also seen in the formation of the concentric corpuscles in the thymus and the spleen. Division of masses occurs plainly in the production of somites, i.e. the metameric segmentation of mesoderm and neural crest. Fusion of masses occurs when two ganglia unite and attachment of masses is seen when the sclerotome and the notochord come together.

Another version of the same catalogue is seen in the table taken from Kellicott. Both of the tables include practically all known morphogenetic processes. But the point to which I wish to direct attention specially here is the possibilities which such classifications hold out of laying bare the physico-chemical processes which accompany the differentiation phenomena. Up to the present time, no chemical experiments consciously directed towards this end have been done, but the body of knowledge which has accumulated about the chemistry of the embryonic body as a whole, and which will be reviewed in the succeeding sections, does make it possible to draw certain conclusions in relation to morphogenesis. These tables of Kellicott and Jenkinson are given here to serve rather as a background for what follows than as a summary of the facts. We have in them an assembly of the various means by which differentiation takes place. Each of those means must have, as everyone would grant, a physico-chemical aspect. We may prefer to say that each of them must have a physico-chemical cause and basis. The main aim of the chemical embryology of the future will be to unravel these causes and their inter-relations.

General increase of thickness and extent is among the simpler problems, and seems to be but a sub-section of the general question of growth. All the discussion of this difficult subject is complicated by the fact that nearly everything that has been ascertained about the physico-chemical constitution and activity of the embryo has been ascertained on the intact organism. It is only in very recent times that the first step has been taken towards the penetration of the internal balance of the embryo, namely, the evaluation of the growth-rates and relative sizes at different times of its constituent organs. But surer knowledge about this is bound to come in the not too distant future. Meanwhile it is simple to picture the processes of perforation, for example, as being due to local controlled autolysis. Here the importance of local variations of intracellular *p*H must not be forgotten, and a study of the intracellular *p*H in a great variety of regions at different times during development would be a most valuable work, especially if it were to be done with methods much better than those which have up to now been employed in the estimation of intracellular *p*H. Buytendijk & Woerdemann have made a good start in this direction (see Section 6·1), and there are other investigations which have a similar theoretical importance, but they will be referred to in their place. The powerful influence of *p*H on cell-division, as in the work of Reding & Slosse and of Vlès, Dragoiu & Rose may, however, be mentioned in this connection. The two tables should be borne in mind throughout all succeeding chemical discussions.

Just as perforations may be probably due to local controlled autolyses, so general disappearances of masses of cells may be due to the same factor operating on a slightly larger scale. This conception is made all the more likely by the work which has been done since 1919 on insect metamorphosis, and which goes to show that there also an autolysis plays a large part. Again, the changes in the form and size of individual cells which may grow larger or smaller may probably depend on the increasing or decreasing concentration of storage materials in them, and here there is an extraordinarily wide field for histochemical methods. Unfortunately these are at present so unsatisfactory that it may be a long time before this aspect of morphogenesis can be even approached. The separation of cells and their fusion into surfaces and layers may also be due to local variations in the sterol and lipoid fractions (tissue constants), the molecules of which might, by changing either their constitution or their position in space or both, be the underlying factors responsible for the process. An immense field lies open here for the application of micro-estimation methods on sections of tissue dissected out from embryos in the early stages of development, a field which has not so far been entered by one investigator.

Processes such as chemotropism, moreover, probably have much importance in morphogenesis. This has been emphasised by Kappers whose work on neurobiotaxis has thrown much light on the early development of the nervous system.

Associated with this conception is that of the localised presence of enzymes. Everything, indeed, depends here on the word "localised". The localisation of substances in the embryo is a study which has not yet begun, for nearly all we know about its physico-chemical life is concerned with it as a whole and with its immediate surroundings. The study of embryonic hormones, for instance, to which much attention will later be given, has not so far been carried on from this point of view, although it does seem clear that the embryo in the early stages is quite devoid of "chemical messengers", and only acquires them later at definite time-points in its development. One may say that the "Nachahmung" school have done much to suggest possible morphogenetic processes without doing anything to find out what they actually are in point of fact. The only means by which this can be done are applications of exact physico-chemical methods, and these will have to be made use of more and more in the future.

We may, however, before leaving this subject take one instance where chemical work has thrown a light on the state of affairs in the young embryo. In 1926 I studied the free and combined carbohydrate contained in the embryonic body of the chick embryo from the 5th day until hatching. Thus 100 gm. of embryo (water and solid together) contained on the 5th day of development 160 mgm. of total carbohydrate and 8 mgm. of free carbohydrate. During development the former falls to about 100, and subsequently rises to over 300; the latter rises all the time in an S-shaped curve to about 48 mgm. This would indicate that the carbohydrate present in the embryo on the 4th and 5th days, at which time there is more in proportion than at any subsequent period, is not in the form of free glucose, and it is certainly not glycogen. The dry weight data gave even more striking results. 100 gm. of dry embryo contain on the 5th day 3000 mgm. of total carbohydrate and about 100 mgm. of free carbohydrate. After that point the total carbohydrate continuously falls, reaching a value of 1750 on the 16th day, while the free carbohydrate continuously rises, its highest point being reached on the 11th day with a value of 360 mgm., after which it falls, but not below 220 mgm. We have therefore a considerable proportion of the sugar present throughout not free and not in the form of glycogen. It is very probable that this fraction can be called "mucoprotein glucose", and that it is present in the form of combination with a protein molecule. Its quantitative relationships are interesting, for it amounts to 2550 mgm. per cent. dry weight on the 5th day, but by the 11th day it has fallen to about 1000 mgm. per cent., after which it remains about steady at that level. No stress is laid on the absolute magnitude of the figures, for the best methods we have for estimating total carbohydrate almost certainly give high results, but there is no reason for supposing that the relative values are not significant.

It seems that this behaviour can be correlated with facts that have long been known to histologists. Von Szíly was the first to describe a cell-free connective-tissue or fibrous ground-substance filling up all the cavities of the embryonic body in the early stages. This has some affinities with the cardiac jelly of Davis, and Baitsell has recently examined its properties with the aid of a micromanipulator. An account of its comparative histology is given by Biedermann. It appears to be secreted by the cells, and provides them with a homogeneous matrix, a kind of natural culture medium in which even migration may take place if and when it may be necessary. As development proceeds, the substance does not disappear, but becomes relatively less and less important in relation to the body as a whole. The nearest equivalent to this ground-substance elsewhere is the Wharton's jelly of the umbilical cord and the vitreous humour of the eye. It is significant that both these tissues are known to be very rich in substances of the mucoprotein type, and the importance of mucoprotein in the beginning of embryonic life leads inevitably to a correlation with the jelly of von Szíly. It explains, or is explained by, the high proportion of combined glucose other than glycogen at that period. This example is intended to give an idea of the kind of correlation between chemical and morphological characteristics which is necessary for the embryology of the future.

Such correlations, in the position in which we now are, are difficult. It is also very difficult to bring together the work of the "Entwicklungsmechanik" school and the results of physico-chemical work, but close attention must be given to it in order to orientate the point of view adopted in this book. Any exhaustive discussion of it is rendered unnecessary in view of the papers by Huxley and Spemann, and the excellent book of de Beer. Other recent reviews of experimental embryology are those of de Beer; Przibram; Mangold; Brachet; Weiss; Gilchrist; Hogben and Schleip.

It will not be possible here to do more than indicate a few of the outstanding problems to which modern experimental embryology has supplied tentative but fairly certain answers.

3·5. Pluripotence and Totipotence

One may first ask, therefore, what answer has been given to the question of organisation in the unfertilised egg-cell, or, in other words, what modicum of preformationism are we still obliged to admit into our conception of the undeveloped egg. In the very earliest stage of development two important critical points occur, or two stimuli are received, the first causing the egg to become radially symmetrical about an axis, and the second causing it to become bilaterally symmetrical in any one of an infinite number of planes passing through that axis. What these processes actually are is entirely unknown, although we can make a guess, as will later be seen. But the fact that they have to occur before differentiation and growth can begin makes it impossible to speak of the spatial arrangement of already differentiated material in the egg, which was the basis of the classical preformationism. It is now correct to say that heredity does not account for the individual but for a number of potentialities, some of which are brought into being in the individual. The only predetermination which exists is the assurance that, if the potentialities in question are brought into actuality, they will produce an organism belonging to the same species as its parents. The application of chemical methods to the undeveloped egg will probably thus in the future not be so histochemical as would have been the case if an arrangement of material had existed there. Nevertheless, what has been said applies to the vertebrate egg only; there are invertebrate eggs, such as those of *Cerebratulus*, which do seem to have an intracellular determinate arrangement. The statement here made may be differently expressed by saying that preformationism is only true in the sense that the constitution of the fused nucleus must have reference to, though it does not actually resemble, the adult form. Protoplasm, and yolk, on the other hand, as Lillie on *Chaetopterus*, Morgan & Spooner on *Arbacia*, and Morgan on *Cumingia* have shown, are homogeneous as far as this goes, since normal development can take place after centrifugation and displacement of visible constituents. (See p. 346.)

It is known that eggs vary a great deal as to their dependence on the arrangement of the initial material in the egg-cell. The extent to which they do so is more or less ascertainable by the extent to which they exhibit totipotence and the rapidity with which they lose it. The phenomenon of totipotence was originally discovered by Driesch in 1900, who found that, if the blastomeres of the sea-urchin's egg were separated at the 2-cell stage, each nevertheless gave rise to a perfect larva of half the normal size. If the blastomere was taken from the 4-cell stage it would produce a larva of a quarter the normal size. Blastomeres from the 8-, 16- and 32-cell stages did not gastrulate well, and not at all if they were derived from the animal pole. No blastomere from the 64-cell stage will produce a gastrula. However, although one blastomere from the 16-cell stage animal pole will not gastrulate, four together will, and will go on to produce a perfect larva, so that the inability cannot be due to lack of specific enteron-forming substance, but simply to too small size. The gradual loss of totipotence, therefore, is an index of the degree to which the egg depends on the localisation of organ-forming materials in the original egg-cell. In some animals the blastomeres are able to regulate their development, if isolated, and produce a perfect larva, in other animals they are not: the former are termed "regulation eggs" and the latter "mosaic eggs". Examples of the former group, besides the echinoderm eggs just mentioned, are the coelenterates, which, according to Zoja, can be totipotent up to and including the 4-cell stage, and the nemertines; but, in this latter case, blastomeres from the 4-cell stage are not perfectly totipotent, but give rise to larvae lacking a few of the usual parts (Zeleny). The group of regulation eggs passes over without break into the group of mosaic eggs. Thus in *Amphioxus*, totipotence exists only at the 2-cell stage, as was shown by Wilson's experiments, and the same applies to amphibia, but only under certain conditions, i.e. if each blastomere contains some of the grey crescent region, which in time will develop into the dorsal lip of the blastopore (Brachet). With the ctenophora we pass to a purely mosaic egg, for, as Fischel has shown, though the normal ctenophore has eight combs, the result of a 2-cell stage blastomere is one with four combs, that of a 4-cell stage blastomere is one with two combs and that of an 8-cell stage blastomere is one with only one. Obviously, then, something was distributed more or less evenly among the blastomeres as the cleavages took place, and cleavage of the cells involved equal cleavage of the comb-forming substance. Crampton has discovered very similar phenomena in molluscan eggs (*Ilyanassa*), Wilson in those of *Dentalium*, Driesch in those of *Myzostoma*, Stevens in nematodes, and Conklin in ascidians. Loss of totipotence may even take place gradually before fertilisation. The organ-forming materials may be actually visible in the undeveloped egg, as, for example, the brownish yellow pigment in *Styela* eggs (an ascidian) which was found by Conklin to be necessary for the formation of muscle. On the other hand, in some cases, as Morgan has shown, pigments and formed elements are present which, while normally distributed to certain cells, can be centrifuged into a different position in the egg without in the least affecting development. Other examples of this have already been given. In 1926 Duesberg found that some of the elements which seemed to be necessary for development, and could not be centrifugally displaced without damage, were mitochondria. But the most significant experiments were those of Jenkinson, who found that normal morphological differentiation could go on in the frog embryo even after the egg had been centrifuged and the fat globules had been made to get into the "wrong" cells. In this case the brain was formed quite normally, though it contained, at any rate as far as histological examination could show, a much greater quantity of fat than usual. Although this process could not be carried beyond a certain point, it did show that fat alone was a structural material rather than a specific organ-forming substance. These regulatory processes occurring after centrifugation were shown by Konopacka for the frog's egg to be remarkably independent of temperature. Totipotence phenomena probably underlie the remarkable cases of Polyembryony which occur sporadically throughout the animal kingdom (cyclostomatous Bryozoa, earthworms, parasitic Hymenoptera, and Armadillos) and which have been reviewed recently by Patterson. The

Armadillo produces four identical quadruplets from one egg, and a single egg of the parasite *Litomastix truncatellus* gives rise to about 1500 individuals.

3·6. Self-differentiation and Organiser Phenomena

The passage of time between fertilisation and hatching can, it has been found, be divided into three periods. These may be summarised thus: (1) division and re-arrangement of pre-existing material and structure (up to the end of the formation of the germinal layers), (2) primary or non-functional differentiation in which the organ rudiments are determined irrevocably and their early differentiation proceeds, and (3) secondary or functional differentiation, in which the inception of active function by the new organs brings about important consequences. "Up to 1910", it has been said, "the principal achievements of experimental embryology could be summarised thus: first, that nuclear division in early development was not differential, the nuclei of the embryo all being equivalent; second, that most fragments of the germ could, before the onset of gastrulation, if of sufficient size, regulate themselves to produce a whole embryo or an approximation to it; third, that in a few cases definite 'organ-forming materials' existed in the fertilised egg—visibly differentiated regions causally correlated with the development of certain organs; and fourth, that Roux's doctrine of the struggle of the parts was valid in the later stages." The intermediate part of development was not, in fact, very well understood. But Spemann's discovery in 1918 that the region of the dorsal lip of the blastopore was a differentiator or organisator, as it was called, threw a new light on the middle period. By grafting a piece of it into another embryo (of different species so as to recognise the parts by their colours) he found that it would cause the host tissues between it and the animal pole to form the primary axial organs, i.e. neural plate, notochord and somites. It would initiate, in fact, the self-differentiation process, and would cause cells which previously would have had the potentiality of becoming almost anything according to their position to set out on their path of irreversible differentiation. Then Brachet showed that the organiser in the dorsal lip region exerted its formative effect behind as well as before. If a cut was made so that continuity between the organiser fragment and the posterior region was interrupted, there would be no formation of notochord and somites behind this obstacle, and, on the other hand, a cut between the organiser and the anterior region would exert the same effect there. The influence evidently demands continuity for its operation.

It is important to distinguish the actual differentiation of this middle period from the invisible determination which foreruns and controls it. Spemann showed, for instance, that in the early neural plate stage, before any sign was present of the optic vesicle, there was a predetermination of the eye as a whole, and also the separate areas for optic stalk, retina, and tapetal layer. Bulbar, ventricular, atrial, and sinus substances are similarly determined before any trace of the heart appears. Huxley proposed the term "chemodifferentiation" for this invisible point of decision as to the fates of cells*. After this point the organism is a chemical mosaic of qualitatively unlike regions, in which regeneration is impossible. "If a gastrula be cut into two", he says, "each half forms only those organs which it would have formed as part of the whole; again, if a piece of the future brain region is cut out of the embryo in the neural plate stage and then grafted back in the reversed position, its different parts still produce the structures that would have been produced in its normal position. Each chemically determined region is separate and irreplaceable." And this state of affairs proceeds until the onset of function, after which, with nervous control and the activities of hormones in humoral channels, a third and quite different period comes into being. It is most important to note, meanwhile, that the point of chemodifferentiation is associated with gastrulation. In the section on resistance and susceptibility a good deal of evidence will be presented which tends to show that gastrulation is one of the main critical points in development, points at which a less degree of external interference is required than at any other, in order to make development go wrong.

The opportunities afforded by the tissue culture technique have also been utilised in the study of the middle period of self-differentiation or irreversible morphogenesis. Obviously the cultivation of an explant of a certain region of embryonic tissue at different stages will (or may) reveal whether it has been determined or not. This determination may from one point of view be regarded as the point where the curve of decreasing totipotence reaches the base-line. For totipotence persisting into later stages, such as blastula and gastrula, demonstrates its presence by the capacity of any piece of tissue to develop into structures which it never would have done if it had been left to itself. The name usually given to this first-period indeterminateness is "plasticity". Thus before gastrulation Spemann found that a piece of epidermis normally destined to fold in to form the ectodermal nerve-cord can be exchanged for a piece of ordinary epidermis with the result that the presumptive nerve-cord will turn into skin and the presumptive skin into nerve-cord. Such plasticity occurs all over the embryo before gastrulation, and Spemann was able, in some very beautiful experiments, to work with the dark-coloured *Triton taeniatus* and the light-coloured *Triton cristatus* together so that dichromic grafted embryos were produced. But if one attempts to make such transformations as these after gastrulation has been completed, one meets with no success whatever—even though no differentiation at all is visible, the hidden process has determined the fate of the cells.

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* We do not know how far this process is really chemical. Decision would be easier if we knew the rate at which, say, medullary plate ectoderm becomes specifically eye-forming substance. Determination-rate is probably at least as important as Growth-rate and Differentiation-rate.

In the preliminary plastic cells even the germ layers are interchangeable. Pieces of presumptive ectoderm can be planted just beneath the dorsal lip of the blastopore, and being carried in as invagination proceeds can afterwards be recognised as taking part in the constitution of mesoderm or endoderm. In other words they behave "Ortsgemäss", i.e. according to the arrangements of their host, and not "Herkunftsgemäss", i.e. according to the arrangements of their origin. Such pieces can be earmarked by the use of vital stains, as Goodale in his work against the concrescence theory was the first to show, and Vogt has in more recent years done many experiments of this nature. The converse, namely, that presumptive endoderm or mesoderm can give rise to ectoderm, has been demonstrated by Mangold, and similar remarks hold with respect to the limb buds.

The fact that the organiser of one kind of *Triton* can bring about its effects in another kind of *Triton* naturally led to the question of what taxonomic distance was necessary to prevent the action of the organiser. Geinitz, working on this point, found that the organiser of even very different species such as *Amblystoma* or *Bombinator* could be grafted into *Triton* embryos with efficient results. The grafts might even come from different genera, families, or sub-classes. Obviously the region of the dorsal lip has in different embryos something very much in common, which can act in a most remarkable way on neighbouring tissues. More extraordinary still, the influence is not a peculiar property of the blastopore lip, for other tissue can be grafted into it, and then can be used as an organiser after having been, as it were, "infected".

We have already seen that the organiser requires contact for the spread of its influence and will not function across a cut. The spread, Spemann found, also takes a measurable time, for at an early stage the ectoderm near the organiser is determined to turn into notochord, while further away it is still indifferent. Ruud & Spemann threw light on the finding of Wilson already referred to when they observed that, if the newt gastrula is divided into halves, only that half which contains the organiser will develop. Brandt has thrown further light on the process of chemodifferentiation and has divided it up into three periods or phases, reversible, critical, and irreversible. Thus different amphibian species which look externally in the same developmental stage may or may not be equally chemodifferentiated.

The influence that radiates from the dorsal lip is not apparently under the control of any other internal factor; it is therefore called an "organiser of the first grade". But some of the organs which differentiate under its influence show in their turn an organising action on the structures developing near them; thus, the axial structures exert an effect on the development of the thoracic and abdominal viscera (*situs inversus viscerum* experiments). They are therefore called, as Spemann suggested, "organisers of the second grade". In the same way the eye-cup is usually, though not always, an organiser of the second grade with respect to the lens, as Spemann; Lewis, and many others have shown.

There is certainly no doubt that some organs at a certain stage contain within themselves all the factors necessary for their complete development as far as the beginning of the third period, while others do not, and seem to depend upon adjacent organs. So far only a few of these relationships have been worked out, but it is very probable that the factor of time is an important one, and, while at one stage an organ or part may be dependent upon some neighbouring second-grade organiser, at another stage it may be quite self-differentiating. Examples of these relationships can be found in the work of Streeter on the amphibian ear, which showed that the membranous labyrinth is self-differentiating, and of Luther, which showed that, on the contrary, the cartilaginous auditory capsule is dependent-differentiating, i.e. in relation to the auditory vesicle as to a second-grade organiser. Again, in *Bombinator* the visceral rudiments are self-differentiatory, according to Holtfreter.

Tissue culture methods have been already referred to as important in the analysis of the differentiatory power of a given anlage. These may be of various kinds. One of the most valuable is the transplantation of the tissues of bird embryos on to the chorio-allantoic membrane of another egg, where it can easily be seen whether or not they continue to develop in isolation, and, if they do, what they develop into. In this way Danchakov found that the blastoderm of the chick develops properly in isolation, but only after some hours. The degree of differentiation here depends entirely on the age of the embryo from which the piece is taken. Thus Hoadley found that pieces of an embryo only 4 hours old will, when transplanted on to the chorio-allantoic membrane, produce an eye with pigment cells only; if the age of the embryo is 6 hours, an eye with pigment and retinal cells will be produced, and, if the age of the embryo is 8 hours, an eye with pigment and stratified retina will result. At 10 hours the primitive groove is formed, and, if a piece be taken from an embryo after the beginning of somite formation, a completely self-differentiating eye will develop on the chorio-allantoic membrane. Other workers have made similar experiments with different regions of the chick embryo as follows: *limbs and limb girdles*—Murray & Huxley; Selby & Murray; and Spurling; *eye*—Barfurth & Dragendorff; *head*—Lillie, and Danchakov; *spinal cord*—Agassiz & Danchakov; *nose, ear and mesencephalon*—Hoadley; *metanephros*—Atterbury; *otocyst*—Fell; *gonads*—Minoura; *mesonephros and primordial germ cells*—Humphrey; *various tissues*—Hiraiwa; *heart*—Danchakov & Gagarin. The other main form of explantation work is the *in vitro* technique of tissue culture. This has been used to study self-differentiation by Reinhov, who found that the kidney of an embryonic chick would develop glomeruli, tubules, and capillaries *in vitro*, and by Strangeways, who got good differentiation in embryonic cartilage. Working with the same technique Strangeways & Fell showed that the limb buds of embryonic chicks have only a small power of self-differentiation *in vitro* (or *in vivo* in transplants), but that, on the other hand, the eye showed remarkable capacities, producing all the constituents of the normal eye, but not growing much in size. And with 6-day femurs, perfect self-differentiation was produced *in vitro* by Fell & Robison. Most interesting of all, Danchakov found, while studying the degree of self-differentiation in various chick tissues, that the mesonephros degenerated after a time in the grafts, just as it does in the intact embryo. A commentary on the relations between tissues acting as second-grade organisers and the tissues they influence is seen in the observations of Champy, who found that epithelium and connective tissue (from post-embryonic stages) in pure cultures by themselves underwent dedifferentiation, and reverted to an embryonic condition. But if they were both present together in one culture the differentiation was maintained, yet only so long as the connective tissue constituent of such a culture was living. If it died the epithelium dedifferentiated. In just the same way the kidney tissue of the mouse grows alone as a sheet of undifferentiated tissue, but can be made to take on its usual character by the addition of connective-tissue cells to the cultures (Drew).

"It will be seen", says Huxley, "that the discovery of the organiser and of the gradient coordinate system enables us for the first time to give a coherent formal account (however imperfect in detail) of the early stages in development. In so doing we sail clear of the difficulty which has beset so many minds of understanding how differentiation can be compatible with absence of qualitative nuclear division. Loeb in his book *The Organism as a Whole* was driven to assume that the Mendelian chromosomal genes were only responsible for minor characters, the main course of development being determined by the ovum, which, owing to its assumed possession of organ-forming materials, was to be regarded as the 'embryo-in-the-rough'. It is now seen that the egg cannot be held to become the 'embryo-in-the-rough' until chemodifferentiation has started. After this moment specific organ-forming substances are all-important, but in most unfertilised eggs they scarcely exist. The production of these organ-forming substances depends upon the varying interaction of organiser and genes in regions of various activity. The differential which determines the variation of activity is the system of metabolic gradients, which, although definitely organised, is very far from constituting the egg an embryo, however much in the rough. One of the two main gradients is determined at or after fertilisation by agencies external to the ovum. The other is determined in the unfertilised egg, but from an analogy with other forms, it is to be expected that this too will be found to have been determined earlier by agencies external to the oocyte (position of the oocyte in the germinal epithelium, blood-supply, etc.)." Such is the general scheme to which the recent researches in experimental embryology have led. It is to be remarked that it includes in intimate association with the working of the organiser the theory of metabolic gradients. It will be necessary to treat this in some detail since it is based on biochemical conceptions, and in some cases biochemical experiments. But, before doing so, some further space must be devoted to the organiser.

What determines the direction of the differentiation induced by an implanted organiser? It might determine for itself the direction in which it would radiate its influence or this might be a function of the host. Geinitz's experiments have shown that the latter alternative is the more probable one. At exactly what stage the cells of the dorsal lip acquire their organising power is naturally very difficult to ascertain, and has not yet been ascertained with accuracy. Moskovski long ago found that, if the grey crescent in the frog's egg was injured by pricking with a hot needle or other means, no embryonic anlage or organ-rudiments would be developed, although gastrulation was not inhibited, and Spemann obtained precisely similar results by eliminating the dorsal (i.e. grey crescent-containing) blastomere at the 2-cell stage. The organiser must therefore originate very early. But Spemann has so far not been able to get a transplanted piece of grey crescent to function as an organiser, although Bautzmann reports success with parts of blastulae.

Of second-grade organisers Spemann says, "The optic vesicle can be regarded as the organiser of the lens. Yet the vesicle itself, as well as any organising power which it may possess, is not evolved through mere self-differentiation of an anlage of an early gastrula: on the contrary, the differentiation of this particular anlage itself is determined by an external stimulus. We have seen that at an early stage of gastrulation the presumptive eye-anlage can be replaced by presumptive epidermis and it would be possible to choose the implant so that it should contain the presumptive lens area. In this case the two ectodermal layers would exchange their rôles—the stimulator becoming the stimulated. The optic vesicle may then be termed from this point of view an organiser of the second grade". The same state of affairs exists between the medullary plate and the roof of the archenteron. Spemann showed that a piece of presumptive ectoderm, e.g. a piece of presumptive medullary plate, could be made to develop into archenteron when implanted into the dorsal lip of the blastopore and allowed to pass inwards with the gastrular invagination. But, on the other hand, the same experiment can be reversed, and a piece from the roof of the archenteron taken, grafted somewhere else and so allowed to induce a medullary plate in any indifferent epidermis. "Geinitz", said Spemann, "recently combined these two experiments into one. A piece of presumptive epidermis was removed at the beginning of gastrulation from an embryo of *Triton taeniatus*, heavily stained with intra-vital stains and implanted into the dorsal lip of the blastopore of another unstained embryo at the same stage of development, in such a manner that it invaginated and formed a portion of the archenteron. It was then removed again and this time transplanted into the cleavage cavity of a third embryo at the onset of gastrulation. In the course of gastrulation it came to lie under the ectoderm and induced in the latter the formation of a secondary medullary plate. This transplant, if left in its normal environment, would have differentiated into epidermis; under the influence of the archenteron—into medullary plate, perhaps into eye-anlagen and would then have induced the formation of a lens. In its third environment it became itself an organiser and induced the formation of a secondary medullary plate."

What is the extent of the region occupied by the organiser? This question has been investigated by Bautzmann, who definitely settled it by transplanting small trial pieces from the whole surroundings into the blastocoele cavity of other gastrulae. Gastrulation brings these under the ectoderm and then their power of induction, if they have any, will manifest itself, as Marx showed, by the appearance of a medullary plate in the overlying ectoderm. The region of the gastrula, then, which may be said to contain the organiser, is a semicircular area above and beside the upper lip of the blastopore. Normally it is invaginated, and gives rise to notochord and mesoderm.

A good deal is known about the structure of the organiser region. It must have an axial longitudinal structure which is not lost by transplantation, for the second embryo may readily be obtained at right angles to the host embryo if the organiser is transplanted into it at right angles. It must also have some sort of "laterality", as has been proved by Goerttler *. Roux long ago concluded from work with half-embryos that each lateral half could develop more or less independently of the other, and Vogt, by subjecting salamander eggs to a temperature gradient by holding them in a silver plate on each side of which water at different temperatures was circulating, found just the same thing. The left half could be made to hypertrophy in relation to the right half or *vice versa.* Huxley made very similar experiments with a less abrupt temperature gradient. These experiments showed that the embryo as a whole has a half-structure. But Goerttler went further in removing the left side of a dorsal lip and grafting a right side, as it were, from another embryo into it; whereupon two right halves of a medullary plate and two right medullary folds were formed. Thus the organiser itself must possess laterality. It has also what might be called a regional structure, in that different parts of one organiser tend to produce different embryonic structures. But the question is an extremely complicated one, for a given part of the organising region tends to induce more than it ought, and there are signs that the term "harmonious equipotential system", which was first applied by Driesch to totipotent blastomeres, may also be required for certain parts of the organiser region at certain times.

Of the physico-chemical nature of the organiser very little is known. The small size of the amphibian material on which most of these experiments have been made, and the body of experimental difficulties as a whole, have made this side of the subject very backward. Heteroplastic transplantations have shown, as we have seen, that the organiser is not species-specific. It cannot act across a gap, but requires continuity of the cell-mass for its effect. The inductive power of the cells of the dorsal lip is not abolished by drying them, according to Spemann, but freezing and thawing does lead to its loss. Mangold has begun some interesting experiments on the quantitative aspects of the working of the organiser.

Whatever is the mechanism of the organiser, there is evidence that it retains its activity unimpaired for a long time. Thus Mangold found that a piece of the brain of a free-swimming larva would still induce a medullary plate in the early embryo, so that the organising power was still present, although it had long been unnecessary. Again, Bautzmann transplanted a piece of notochord from a neurula into the blastocoele cavity of a much younger embryo, and found that the notochord fragment induced a medullary plate in the ectoderm above it. It must, then, have retained the power to do so long after it had been necessary to exercise it in the process of normal development. Precisely analogous are the experiments of Wachs, who found that regeneration of the adult amphibian lens takes place under the inducing influence of the retina, just as had happened originally in ontogenesis. Such experiments demonstrate that the organiser persists into post-embryonic life.

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* Yet Spemann has found the inductive power still present in squashed pieces from the organiser region. Does this mean that the laterality of the organiser is stereochemical rather than cytological?

Spemann & Mangold also found that, when they transplanted a piece of medullary plate into the blastocoele cavity of an ungastrulated embryo, it would induce in its turn another medullary plate. This process, which they called "homoiogenetic induction", is really a special case of the action of a second-grade organiser, in which a tissue produces a replica of itself.

A process occurring in the first and second periods of embryonic development, which has not so far been touched on, is that of "double assurance". Thus the eyeball in amphibia may induce a lens in foreign epidermis, but in some amphibia the lens may develop on its own in the absence of any eyeball, i.e. is self-differentiating, and not dependent on the action of the optic vesicle in its capacity of second-grade organiser. In *Rana esculenta* both faculties have been conclusively shown by Spemann and Filatov to coexist. Probably further analysis of development will show that this double assurance principle plays a great part in morphogenesis, and that cells only become what they do under the influence of as many as three or four contributing causes*. The double assurance principle may correspond to the factors of safety which appear in structural engineering, so that, if one process goes wrong, the embryo can still manage to complete its development with the aid of the others.

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* Thus Bautzmann got differentiation of medullary plate from fragments of *Triton* blastulae which lacked the entire organising region. Similarly Hoadley got self-differentiation of parts of the chick blastoderm at 4-6 hours, whereas Waddington showed such parts to be still plastic up to 18 hours or longer. It is clear that chemical determination is often controlled by more than one agency, and that the times of activity of these agencies may overlap.

It is to be noted that all the experiments which have been described have been carried out on amphibian material, but the evidence which leads us to see in them a validity over all types of embryo, even mammalian ones, is rapidly accumulating. Seidel has extended the concept to insect eggs, while von Ubisch; Runnström; and Hörstadius find evidences of it in those of echinoderms, Wilson in those of annelids, and Gräper; Hunt and Waddington in those of birds. It is highly probable that organiser phenomena will in time be found to exist in all varieties of embryo. The division of embryonic development into three main periods, however, is more generally certain, and may be taken to hold in all cases.

As we have already seen, the junction between the first and second periods of development varies with different eggs. In some (regulation eggs), the point of chemodifferentiation does not occur till gastrulation—this is its latest point—but in others it occurs earlier, at some time during cleavage and blastula formation, while in pure mosaic eggs, of which few are known, it occurs before fertilisation. The duration of the second period, the period during which irrevocable differentiation is going on, is rather variable. It ends with the beginnings of function on the part of the foetal organs.

3·7. Functional Differentiation

In this third period further differentiation may be dependent on functional activity for its proper progression. The classical example of these mechanisms is the circulatory system, in which Oppel & Roux found that the structure and constitution of blood-vessels depended largely on how they were being utilised by the circulation as a whole. Fischer & Schmieden, for instance, transplanted a section of vein into the course of an artery, where it acted perfectly well, but took on the characteristics of an artery, i.e. its connective tissue content increased and its muscular walls were more than doubled in thickness. Exactly the same thing happens with regard to the central nervous system, and to the bones. "The formation of the normal structure of the bones", concluded Landauer, "is caused largely by the static conditions of muscle tonus during embryogeny." Dürken's well-known experiments may be mentioned, in which, when the hind limb buds of a frog embryo are removed, the hind brain does not develop normally. Again, Babák observed that in tadpoles the area of the active intestinal absorptive surface is directly proportional to the amount of vegetable matter in the diet; if it is large, the intestines are capacious and long, if it is small, the intestines are small. In this connection the case of the changes in the intestinal tract of the opossum studied by Heuser is of interest, for it has a gestation time of only 13 days, and has to live some time on milk in the pouch. Babák's work was criticised by Klatt, but has been confirmed more fully by Elven.

The three periods in the life of the embryo are now known to have different relations to regeneration. Przibram's "law of apogenesis" holds true in the main, namely, that the younger an animal the greater are its powers of regeneration (cf. the work of Abeloos on *Planaria dorotocephala*). But this must be qualified by the statement that, during the intermediate period, no regeneration is possible. Each individual part and organ of the embryo has its work cut out to differentiate into its destined form, and any replacement of lost parts cannot be made. Thus Spurling found that the limb buds of the chick cannot be reformed during this middle period. But in the later period of functional differentiation, regeneration is possible, as has been shown by Olmsted; Morgan & Davis; Davidov; and Nussbaum & Oxner. Perhaps the best study of this is the work of Mackay, Mackay & Addis on compensatory hypertrophy after unilateral nephrectomy. Their figures were as follows:

Age in months	% hypertrophy
1	52·6
3	36·7
6	32·8
12	32·2

It is in this later period of functional differentiation that Roux's doctrine of the struggle of the parts has its significance. The signs of this equilibrium between organs and tissues do not emerge except when the organism is subjected to the stress of an unfavourable environment. Then some parts will be found to have the preference and to take a relatively greater share than the others in the available food-supply. The reproductive glands have an important position here, e.g. the testis of the starved rat in Siperstein's work, and there are many instances where the ova in course of preparation draw to a great extent upon the remainder of the body. A discussion of these facts will be found in the appendix on the maturation of eggs; here it may suffice to mention the work of Greene on the salmon, who found a remarkable constancy in the chemical composition of the ovaries, although that of the rest of the body was varying considerably according to the food eaten.

3·8. Axial Gradients

It is now time to turn to another aspect of the analysis of embryonic development, the theory of axial gradients. A convenient transition is afforded by Bellamy's discovery that the dorsal lip of the blastopore and the animal pole in the frog's egg are regions of "high protoplasmic activity". According to the theory of axial gradients, this would mean that the metabolic rate at those points—obviously of great importance as being the seat of the organiser—was higher than anywhere else in the embryo at that moment. It is plain that this is a matter of much interest, and it is therefore necessary to examine in some detail the theory of metabolic, axial or physiological gradients as a whole. Much of the evidence on which it is based is to be found in the four books of Child, and Abeloos and Ranzi have written valuable reviews of the subject.

The fundamental conception lying at the base of these views is that of axiate pattern. Child emphasised in all his work the idea of polarity and of gradients of activity between poles. He proposed that we should think of the embryo or the animal as existing in a three-dimensional graph or co-ordinate system, and being constituted in a kind of pattern of axes of symmetry. Each axis would pass from one pole to another, but along its length the protoplasmic activity would not be constant; on the contrary, it would be very high at one pole and very low at the other, dwindling away at a definite and measurable gradient. Such an axis may or may not at its origin in time have a visible morphological outward sign of its existence. It may or may not last as long as the differential growth to which it gives rise. In fact, ontogenesis from this point of view is the clothing of the original protoplasmic axiate pattern with a corresponding morphological axiate pattern. The anatomical gross differentiation of parts with reference to a given axis is preceded, as it were, by the appearance of a gradient of physiological activity along this axis. By a gradient of physiological activity Child meant a series of quantitative differences between the properties of the cells, following a definite orientation with reference to the eventual pattern of the animal. Child may be said to have transferred those co-ordinate diagrams which d'Arcy Thompson used to demonstrate animal form from appliances of convenience into descriptions of actual fact, and to have substituted for morphological axes, axes of physico-chemical difference. Difficulty was bound to arise when some sort of identification of the physico-chemical differences was attempted, and Child, content with a loose and general association, chose metabolic rate as the physico-chemical variable. "Axial gradients have often been called metabolic gradients", he said, "because differences in metabolism, or, more specifically, of oxidative metabolism, as indicated by various experimental methods, appear to be characteristic and conspicuous features of them." It will be necessary presently to examine the evidence on which this statement is based, but first of all a few theoretical remarks require attention.

That gradients of various kinds exist within the developing embryo has long been known. The "law of developmental direction" (Jackson and Scammon) or of "cephalocaudal differential growth" (Calkins), which we have already discussed in relation to the growth of parts in the embryonic body, is simply, after all, a statement of the fact known in general to Aristotle, that the head end of an embryo develops quicker than the tail end. Again, as Minot showed, the cephalic somites develop before the more caudal ones. "The first parts to become morphologically visible", as Child puts it, "are the apical or anterior regions, and these are followed in sequence by the successively more posterior or basal parts." Again, there are dorso-ventral gradients. In those bilaterally symmetrical invertebrates which have a ventral nerve-cord (including most worms and arthropods) the ventral and median regions of the embryo at any given level of the body develop more or less in advance of the dorsal and lateral regions. On the other hand, in vertebrates, where the nerve-cord occupies a dorsal position, the dorso-ventral gradient runs the opposite way, and differentiation and growth proceed more rapidly in the median dorsal region than in the lateral and ventral regions. The antero-posterior gradient, however, is the same as in the invertebrates. For a recent discussion of the law of cephalocaudal differential growth Kingsbury's papers should be consulted.

Again, the rule which has been named after F. M. Balfour, that the rate of cleavage in an embryonic region is inversely proportional to the amount of yolk which the cells in it contain, is associated with the gradient system of the egg. For the apicobasal gradient in the amphibian egg, for instance, leading from the protoplasm-rich rapidly dividing animal pole to the yolk-rich slowly dividing vegetal pole, has an enormous effect on the type of development which takes place. It is plain, too, that much more might be said about the relation of gradients to the various classes of eggs, alecithic, telolecithic, and centrolecithic. In 1905 Morgan advanced the hypothesis that the gradation of materials in the egg was a factor in establishing physiological polarity, and Boveri came to very similar conclusions about the *Ascaris* egg.

Child made a step forward from these simple facts when he propounded the conception of primary protoplasmic gradients of which the morphological gradients were the obvious result, for he brought the subject to some extent nearer the point where physico-chemical analysis could begin. Moreover, he was above criticism when he did not specify what sort of physico-chemical activity it was that was responsible for the gradient. But it is difficult to follow him when he concludes that the gradient is one of "oxidative metabolism", "oxidising power" or metabolic rate. Apart altogether from the fact that the experimental evidence will not carry this conclusion, there are theoretical difficulties involved in it. It seems to have its roots, indeed, in that now abandoned idea, which was once common among the followers of Jacques Loeb, that oxidation-processes and growth were very closely allied, even that the master reaction of the growth-phenomenon was an oxidation. If no physiologist now adopts this notion it is because so many researches have shown it to be false. The work of Crozier and his school on temperature characteristics might be mentioned, in which the μ value for growth in general and embryonic growth in particular practically never turns out to be 16,000. Murray's demonstration of the diametrically opposite course taken by growth-rate and metabolic rate, again, is an instance of the same thing. All the tendencies of recent years have been against any close identification of oxidative processes with growth. A little reflection is enough, moreover, to convince one that such an association is far from being *a priori* necessary. In the case of the embryo of the chick in the egg, for example, its increase in size could be represented by the curve *a–b*, in Fig. 95. At the beginning and at the end of the period *x–y* the size of the embryo is of course given by the height of the curve above the abscissa, but to conclude that the amount of solid absorbed by the embryo during that period was equal to the difference between the size at *x* and the size at *y* would be to assume that the efficiency of the embryo was 100 per cent., which is certainly not the case. As the diagram shows, what has actually happened could be represented in abstract form by a peaked curve superimposed on the growth-curve representing by its upward sweep the total quantity of solid absorbed during the period in question and by its downward fall the total quantity of solid catabolised, or "oxidised", and excreted as carbon dioxide, uric acid, etc. The fact that the downward slope does not go down as far as the upward slope has come up is what makes growth possible. The process of growth, then, might be related to that of catabolism as rival, not as offspring, and instead of resulting from it might compete with it for the available solid substance. Or, on the other hand, the steeper the curve the higher the peaks might be above it, in which case metabolic rate would be highest when growth-rate is highest. Everything depends on the relative magnitudes. The experimental fact that the metabolic rate of the whole embryo is highest when the growth-rate is also highest reveals no simple causal nexus between them, and it is not theoretically correct to assume such a relation.

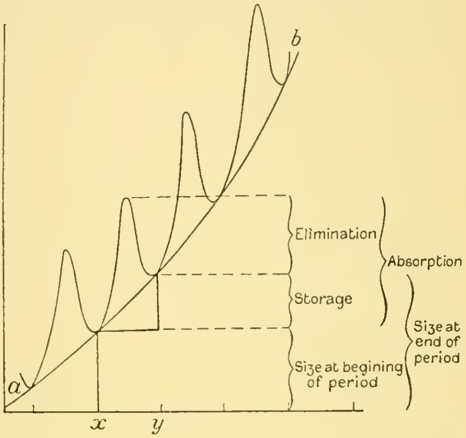

Fig. 95.

Child has used various methods to ascertain the existence and distribution of his gradients:

(1) Direct susceptibility.
(2) Indirect susceptibility.
(3) Reduction of potassium permanganate.
(4) Formation of indophenols.
(5) Observation of electrical potential.
(6) Estimation of carbon dioxide produced.
(7) Estimation of oxygen taken in.

It is clear that the only methods capable of informing us whether gradients of metabolic rate are involved (cubic millimetres of oxygen used up per gram per hour or cubic millimetres of carbon dioxide given off per gram per hour) are direct estimations of these gases. Yet out of the hundred odd papers which make up the core of the literature on physiological gradients, not more than a dozen at the very outside are concerned with these fundamental measurements. In 1915, when Child's first two books were published, the only evidence available was due to Tashiro, who had, at Child's request, examined the behaviour of planarian worms in his microrespirometer, and had concluded that the pieces from the cephalic end gave off more carbon dioxide relatively than those from the caudal end, though the figures seem never to have been published. In view of the criticisms which Adam; Bayliss; and later Parker brought against Tashiro's apparatus, not much weight can be attached to these results.

In 1921 Robbins & Child obtained evidence from a study of regeneration in planarian worms that the larger amount of carbon dioxide was produced relatively at the head end, and in the following year Hyman & Galigher reported the same relationship to hold as regards oxygen for the oligochaete worms *Lumbriculus inconstans* and *Nereis virens*. Preliminary results on *Corymorpha palma*, a large tubularian hydroid, were given by Child & Hyman to the American Zoological Society in 1922 and 1923, and published *in extenso* by Child & Hyman in 1926. Hyman extended this to oxygen uptake of planarians in 1923. Her results were paralleled by figures for carbon dioxide production estimated by a colorimetric method in which the time taken by pieces of the stem to reach a definite acidity was measured. But no precautions were used to ensure that the acidity measured was due to carbon dioxide and not to other acids, for the method did not involve passing a stream of air through the water containing the stem under investigation. The oxygen determinations, on the other hand, were all done by the Winkler method, and were never checked by any differential manometer technique. That this is a serious deficiency is evident from the remarks of Shearer, who in a private communication says, "With Haldane's and Barcroft's apparatus I could not get any results which even begin to support Hyman's tables. I think that the Winkler method is useless where a lot of slime is discharged into the water (as is the case with Planarians)". This view finds many supporters among those familiar with the Winkler method.

Hyman and Child made an attempt to gauge the concentrations of glutathione at the different levels of the stem of tubularians, using a modification of the nitroprusside test, from which they concluded that glutathione gradients were present. Such a statement, however, cannot be accepted in the absence of exact quantitative data, especially considering the unspecific character of the nitroprusside test applied to an intact animal.

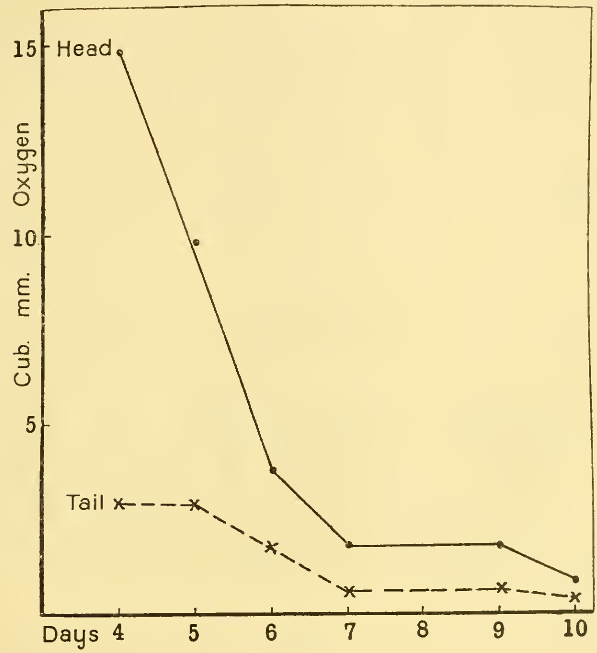

Fig. 96.

Apart from these rather unsatisfactory researches, nothing has been done by Child and his collaborators on the direct verification of their theory. In spite of this, they carried over the conception of metabolic gradients to the developing embryo without modification, and the question naturally arises, to what extent were they justified in doing so? The position is, in a word, that it cannot yet be considered as proved that gradients of metabolic rate exist, much less that they accompany gradients of other entities, such as susceptibility and electric potential, even in the case of invertebrates—tubularians, planarians, annelids, etc. Are we then justified in asserting, direct evidence being absent, that a similar relation holds for vertebrates and embryos in general? Hyman's few figures for respiration of *Fundulus* eggs tell us nothing about the metabolic rate, for no weighings of embryos were made. As regards the embryo, it is most unfortunate that the only other piece of relevant evidence is contradictory. Shearer in 1923 made an investigation of the oxygen consumption of the head end and the tail end of chick embryos, using the Barcroft differential manometer. Fig. 96, taken from his paper, shows the results which he obtained. On the 4th day the head pieces took up more than three times as much oxygen per gram per hour as the tail pieces did, but by the 10th day the two curves had almost come to coincide. Both of them fell, showing that the metabolic rate of the cells was declining with time in the usual way. Such a graph fits in very well with the morphological picture, for by the 10th day the axiate pattern has long been established, and subsequent development mainly concerns growth in size. Shearer went on to investigate the action of acetone powders in order to see whether the higher metabolic rate of the head pieces was dependent on structural conditions. The powders were made in the same way as acetone yeast preparations by first dehydrating the tissue in acetone, and subsequently desiccating completely. Such powders on being made into a thin emulsion with distilled water respire. The results were as follows:

c.c. oxygen taken up by embryos of 6–7 *days' development per amount of tissue containing* 1·4 *mgm. nitrogen.*

Exp.	Head	Tail
1	0·62	0·23
2	0·52	0·29
3	0·47	0·27

This then afforded a clear demonstration in favour of the identification of metabolic rate gradients with axial gradients, and gave evident support to Child's views. Unfortunately, in a later statement Shearer reported that he had not been able to repeat these findings, and that further experiments had very much modified the original conclusions. "I have since concluded", says Shearer in a private communication, "that I was dealing with the rate of cytolysis undergone by the head and tail fragments. The younger the embryo the more readily the tissues disintegrated and cytolysed during an experiment. The heads containing the large watery brain vesicles cytolysed very quickly and gave a wholly abnormal respiratory rate, but the tail fragments, being composed of less delicate material, did not undergo cytolysis so quickly. Whenever you get any cytolysis the oxygen consumption is of course greatly increased." Shearer afterwards extended the work with Barcroft microrespirometers to planarian head and tail fragments, and absolutely failed to get the results which should have been found on the Child theory. Here the position was complicated by muscular movement which did not go on to the same extent in the head and tail fragments. The situation is therefore at present a deadlock, and we are at a standstill until further accurate work on the lines already laid down by Shearer is carried through.

Practically no importance can be attached to the experiments which have been made with potassium permanganate and indophenol blue. Child & Hyman in 1919 placed embryos in very dilute solutions (*M*/10,000) of potassium permanganate, and described in all cases a gradient along the cephalocaudal axis with maximum activity, i.e. maximum reduction of the permanganate, at the anterior end. Similar work was afterwards done by Child (on *Corella*), by Galigher, and by Hyman. As a demonstration of contributory interest the permanganate method has its value, but it is far too uncertain biochemically to serve as the basis for the identification of axial with metabolic gradients in embryos. As for the indophenol blue reaction, Child applied it to the development of the starfish egg, and observed that the apical third or half of the body of blastula and gastrula stages was always stained a deep blue before the blastopore region had become stained at all. In his 1924 book he stated that exactly similar gradients had been observed with methylene blue, reduction of this dye being faster at the cephalic than at the caudal end of the embryo. These observations cannot by any means permit of conclusions about gradients of metabolic rate.

These criticisms of one of the main aspects of Child's theory are essentially the same as those made by Parker and by Loeb in his book on Regeneration. "The unit for the measurement of metabolism", said Loeb, acidly, "is the calorie, and the calories produced by an animal or by one of its segments are not measured by the time required to dissolve the animal or one of its segments in a solution of potassium cyanide." Nevertheless, the susceptibility method does show up the existence of gradients of something. The very numerous studies of Child; Hyman; and Bellamy on the susceptibility of different regions of embryos at different stages are quite sufficient to prove that. They will therefore be called in the remainder of this discussion axial or physiological gradients, and their nature will be left undefined. An example not tied up with any theory of the nature of the gradients is the staining gradient of the chick embryo found by McArthur to hold for many acidic and basic vital dyes. It must be admitted that throughout Child's treatment of the subject he confuses growth-rate with organisation- or differentiation-rate though the two are certainly not identical. In fact, it is often difficult to tell from Child's arguments whether he means growth-rate, differentiation-rate or metabolic rate, and it is not very helpful to lump them all in one as "level of physiological activity". But it is time to come to the facts obtained by the use of the susceptibility method.

Child used two variations of the susceptibility method, the direct technique and the indirect technique. In the former case the resistance or susceptibility is determined directly by concentrations of toxic agent which kill the animals within a few hours. For a particular species a concentration must be determined which kills without acclimatisation, but which does not kill so rapidly that no differences between parts of the body are discernible. Child has used all kinds of toxic agents in his experiments, various cyanides, alcohol, ether, chloroform, chloretone, acetone-chloroform mixtures and other narcotics, X-rays, ultra-violet rays, ammonia, soda. He has obtained the best results with those substances which have a narcosis time and a killing time very close together, in other words, with those substances whose effects are not complicated with narcosis. The most favourable poisons, he found, were the cyanides. As Hogben has pointed out, it would have been far more favourable to the metabolic gradient theory if Child had confined his attention to the cyanides, which are known to have an inhibitory action upon some, though not upon all, tissue oxidations. For then it would not have been known that substances such as ether and chloroform had precisely the same effects, and the association between axial gradients and oxidation-rate would have been more convincing.

Since the death and disintegration of the different parts of the body usually follow a regular time sequence, Child found it possible to determine the time not merely of disintegration of the whole animal, but of various regions of the body. The method used as a rule was to examine the lot of animals at intervals of half-an-hour and then to record the condition of each individual. Arbitrary values having then been attached to the various stages of disintegration, curves can be constructed showing the rate at which the process has gone on. Fig. 97, taken from Child's 1915 book, shows such a curve constructed for specimens of a flatworm, *Planaria dorotocephala*, the curve *ab* representing the susceptibility of young and the curve *cd* representing that of old animals. The correlation between age and susceptibility should be noted.

The indirect method involves the principle of acclimatisation. In general, according to Child, the ability of an animal to acclimatise itself to cyanide or other toxic agent varies with its metabolic rate, or rather its level of physiological gradient. The ability of parts of animals to become acclimatised also alters with the same variable, so that the indirect method affords a way of estimating gradients in the embryo. The relation between age and survival time in solutions of the concentration necessary for the indirect method is exactly the converse of what it was in the direct method, for here the younger animals with the higher metabolic rate live much longer than the older ones with the lower metabolic rate.

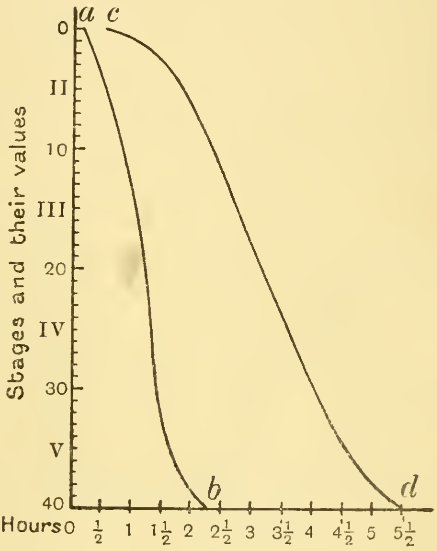

Fig. 97.

The susceptibility method is obviously a very complicated one, and conclusions from experiments with it have to be drawn with caution. In lethal doses which are not concentrated enough to produce death within a short time after the beginning of the exposure, the regions of higher activity are always affected first, so that above a critical concentration susceptibility varies directly as the physiological activity, while below this concentration the reverse of this relation is seen, in that regions of higher activity recover and adjust themselves to the reagent more successfully than regions of lower activity. "In applying the susceptibility method to embryonic development, lethal concentrations may be used but not allowed to act long enough to produce death in the embryo, and in such cases they will, according to Child's interpretation, inhibit regions of higher activity to a more marked degree than regions of lower activity; while on the other hand, in very low concentrations of the reagent such as to permit acclimatisation and recovery, the region of higher activity will be inhibited, according to Child's interpretation, less than regions of lower activity." A simple instance of the operation of Child's conceptions is the case of the eggs of some polychaete worms, *Chaetopterus* and *Nereis*, which, when placed unfertilised in lethal solutions of cyanide, exhibit a progressive dissolution from the anterior end. As development proceeds, the region of maximum susceptibility shifts round to the posterior region (where growth is most active) so that, when the larva is ready to metamorphose, the posterior region is the region which succumbs most readily to lethal and recovers most readily from sub-lethal concentrations of cyanide.

Child's work on the eggs of the starfish, *Asterias forbesii*, brought out the fact that the susceptibility gradients in the unfertilised egg were connected in some way or other with the mode of attachment of the individual egg to the parent body in the ovary, obviously a very important point for the question of the origin of polarity and axial symmetry in the developing embryo. Wilson & Matthews showed that the region where the nucleus lay nearest the surface became in the starfish's egg the apical or animal pole, and Child found that it was from that point on the surface of the egg that disintegration began. From the behaviour of nuclei which had been extruded during cytolysis in cyanide, Child concluded that the nuclear susceptibility gradient ran in the same direction as the cytoplasmic susceptibility gradient. The direction of the axis, he concluded, was determined by the eccentricity of the nucleus. Child found that during the earlier cleavage stages the general gradient was obscured to a great extent by individual gradients in the blastomeres and other incidental factors, and that "in the early spherical blastula before movement begins the disintegration gradient is distinct, but the difficulty in identifying the animal pole and embryonic axis makes it impossible to demonstrate that the gradient coincides with the axis". But "in later free swimming stages of the blastula the direction of movement with the apical region, the animal pole, in advance, and before gastrulation the elongation of the embryo in the direction of the axis and the increasing thickness of the cellular layer toward the vegetative pole render orientation possible at a glance. In these stages the disintegration gradient is very distinct. It begins at the apical end and proceeds with a definite course along the embryonic axis, ending in the region of the vegetative pole where the gastrular invagination will occur". The susceptibility gradient in the gastrula of the starfish Child found to be very similar to that in the blastula, being greatest in the region of the apex and least in the region of the blastopore. Moreover, the gradient in the archenteron wall was exactly the same as that in the body-wall, the apical end of the endodermal invagination being the region of greatest susceptibility. After this time the gradients become less and less distinct until by the bipennaria larva stage they have faded away almost entirely.

Hyman extended in 1916 the observations of Child on polychaete eggs to those of a microdrilus oligochaete, *Tubifex tubifex*. She found that, in the stage when the embryo has begun to elongate, its posterior region was the most susceptible to cyanide, and the susceptibility decreased as one passed forwards. In later stages the head end became more susceptible, and finally exceeded greatly the tail end in susceptibility, so that at hatching it was much the most easily disintegrated region. Thus the posterior region of high physiological level which is characteristic of the adult annelid arises very early in development. After hatching, a worm placed in cyanide disintegrates first at the head end, later at the tail end, so that the two waves of disintegration reach and fuse at a point posterior to the middle of the worm's length. Figs. 98 and 99, taken from Hyman's paper, show the degeneration of embryos of different stages in cyanide.

Child and his associates frequently correlated their susceptibility gradients with gradients of electric potential. Hyman & Bellamy in 1922 gave a full account of the work on this subject with a critical discussion, and at the same time reported their results for frog embryos. Hyde had previously found the heads of recently hatched toad tadpoles to be galvanometrically negative to the heads, and this Hyman & Bellamy confirmed for the frog. "The idea is advanced", they said, "that differences of potential in organisms, particularly the permanent differences which exist along the main axes of animals, are due to differences in metabolic rate at different regions, the region of highest metabolic rate being the most negative in the external circuit, most positive in the internal circuit." In the tadpole, therefore, the highest physiological level appeared to lie towards the tail.

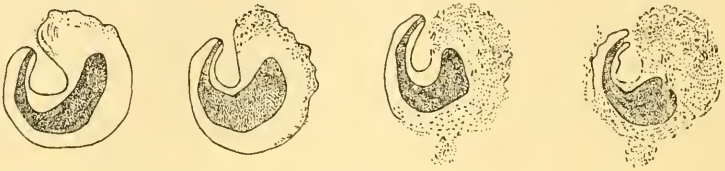

Fig. 98.

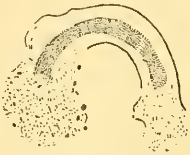

Fig. 99.

Hyman has published a series of papers on the susceptibility gradients of vertebrate embryos. The first of these she devoted to the teleost embryos (*Fundulus heteroclitus* (minnow), *Ctenolabrus adspersus* (cunner) and *Gadus morrhua* (cod)). She showed that, in addition to the primary gradient of the embryo which has its high level pole at the anterior or cephalic end, there were also other "secondary" gradients arising from regions of high susceptibility other than the anterior end. Certain organs, also, may have their own axiate pattern, notably the heart.

The cod and cunner embryos were studied with cyanide in the usual manner, but the impermeable egg-membranes of the minnow made this impossible, so that ammonium hydroxide had to be used instead. In the early blastoderm stages of the cunner and the minnow, the central cells were observed to be the most susceptible, and from them disintegration proceeded to the periphery of the blastoderm. But in the case of the cod exactly the reverse relationship held true;

the periphery of the blastoderm was more susceptible than the central part. In the later blastoderm stages of the cunner the region of high susceptibility is shifted posteriorly, and a certain area along the margin of the blastoderm succumbs very readily indeed to the toxic agent. This is exactly where the embryo is about to arise. The eggs of the minnow could not be examined at this stage. In the cod, the germ ring was always much more susceptible than the central part of the blastoderm, and at its circumference one region is more susceptible than the remainder. This is where the embryonic shield originates, so that the conditions in the cunner and the cod are now very similar. As the embryonic shield grows forward, its anterior margin is most susceptible, and disintegration extends posteriorly from this.

Slightly later stages, when the embryo is visible in the centre of the embryonic shield and the germ ring has advanced more than half-way over the yolk, were not observable with certainty in the cunner and the minnow. But in the cod they were clear enough, and here toxic action obviously began at the anterior end of the embryo, spreading backwards towards the posterior margin of the shield. Still later, at the time of closure of the germ ring, disintegration gradients were observable with ease in all three species, and always the susceptibility was highest anteriorly, diminishing and spreading backwards. The eyes are not very susceptible, and do not degenerate until the wave has passed half-way back along the neural tube. After the germ ring has closed, a secondary region of high susceptibility appears at the posterior end of the embryo. From this point onwards, there is no change in the gradients; there is a powerful spreading backwards from the cephalic zone of high susceptibility and a slight spread forwards from the caudal zone, with no complicating factors. The minnow differs from the cunner in possessing the two zones from the very earliest stages, and at certain early points in development, the posterior zone is the more important of the two. But as development proceeds the posterior zone declines in susceptibility and the anterior one increases, especially after the arrival of the optic vesicles, which fall an exceedingly easy prey to the toxic agent. In very late stages in the minnow, a region of high susceptibility develops in the hind brain where the cerebellum is forming. By this time the tip of the tail has become free from the yolk and somewhat more susceptible again. The cod embryo behaves in much the same way as the other two in the later stages, always having two regions of high susceptibility, the anterior preceding before the closure of the germ ring and the posterior preceding afterwards. Minor variations in susceptibility of eyes, fore brain, etc., were noted. The somites in all three teleosts disintegrate from each end, but more from the anterior than from the posterior. Special observations were made on the heart gradients, which agreed in many particulars with subsequent work by the same author on the gradients of the embryonic heart of the chick, e.g. the venous end was the more susceptible, and the gradient decreased towards the arterial end.

Hyman was able to draw several conclusions from this work important for pure embryology, such as that, in different teleost embryos, the amount of material contributed to embryo formation by the germ ring is variable, being very little in the cunner and considerable in the minnow. This reconciled the views of older workers, such as Morgan; Sumner; and Kopsch. These points, however, together with the fact that her results gave no support to the concrescence theory, are not so important for the present purpose as the delineation of the regions of high susceptibility for comparison with other embryos. The double gradient (anterior and posterior zones of high susceptibility) is also regarded by Child & Hyman as important for a comparison which they make between segments in segmental animals and separate individuals, suggesting that, whereas in annelids the posterior zone is permanent and never comes under the control of the anterior zone, in vertebrate embryos it eventually dies away, so that further segmentation ceases. This need not, however, detain us here.

In the same paper as has already been mentioned, Hyman measured the rate of oxygen consumption of *Fundulus* eggs during their development. Unfortunately, the figures do not give us any information which would either support or weigh against Child's theory of metabolic gradients. Owing to the small size of the embryo, the determinations had to be expressed in relation to 1000 eggs (i.e. embryos + yolks), and, as we have no idea how the wet and dry weights of the eggs or the embryos were varying during this period, we cannot calculate the metabolic rate. This work will be discussed in detail in the section on the respiration of the embryo. Hyman also made a summary of the teratological results which had been obtained by various workers on *Fundulus*, and concluded that in all cases the malformations produced most easily were those of the fore brain, the head in general, the sense organs, especially the eyes, the heart, the circulatory system, and the tail. These results obviously fitted in very well with those appearing from the use of the direct susceptibility method of Child, and this outcome of the physiological gradient conception is perhaps one of its most attractive aspects, for no other point of view serves to account for so many of the facts of teratology. It was long ago pointed out by Dareste that no relation seemed to exist between the application of a certain physiological or physical condition and the resulting teratological modification. Thus Herbst's "lithium larvae" and Stockard's "magnesium embryos" have been shown to be obtainable with a great variety of agents. There is, we may say, practically no specificity in teratological action. "Any type of abnormality", as Bellamy puts it, "may be produced under the influence of any inhibiting agent by controlling the concentration or intensity of action, the length of exposure, and the stage, i.e. physiological condition, of the egg or embryo or parts of the egg or embryo when exposed." Since the differences then do not reside in the teratological agents employed, they must do so in the embryo itself—an admirably Kantian conclusion, which can only be explained on some basis which maps out the embryo into a logical system. The only basis we have is the conception of physiological gradients. It is difficult also to imagine any other view which could explain such instances as the production of the usual terata by fertilising eggs with foreign or injured spermatozoa or treatment of the eggs before fertilisation as in the experiments of Gee. Full discussions of the teratological literature and interpretations of it from this physico-chemical standpoint will be found for the fish embryo in the paper of Newman, for the chick in the paper of Hyman, and for the frog in the paper of Bellamy. It is very noteworthy that new teratological modifications not before obtained have been predicted on the basis of physiological gradients, and have afterwards been verified.

Hyman next studied the gradients during the development of the brook lamprey, *Entosphenus appendix*, an organism of considerable interest, in view of the fact that, like the amphibia, the cyclostomes are one of the three vertebrate groups which develop by holoblastic unequal cleavage. Alcohol and acetic acid were used as the reagents

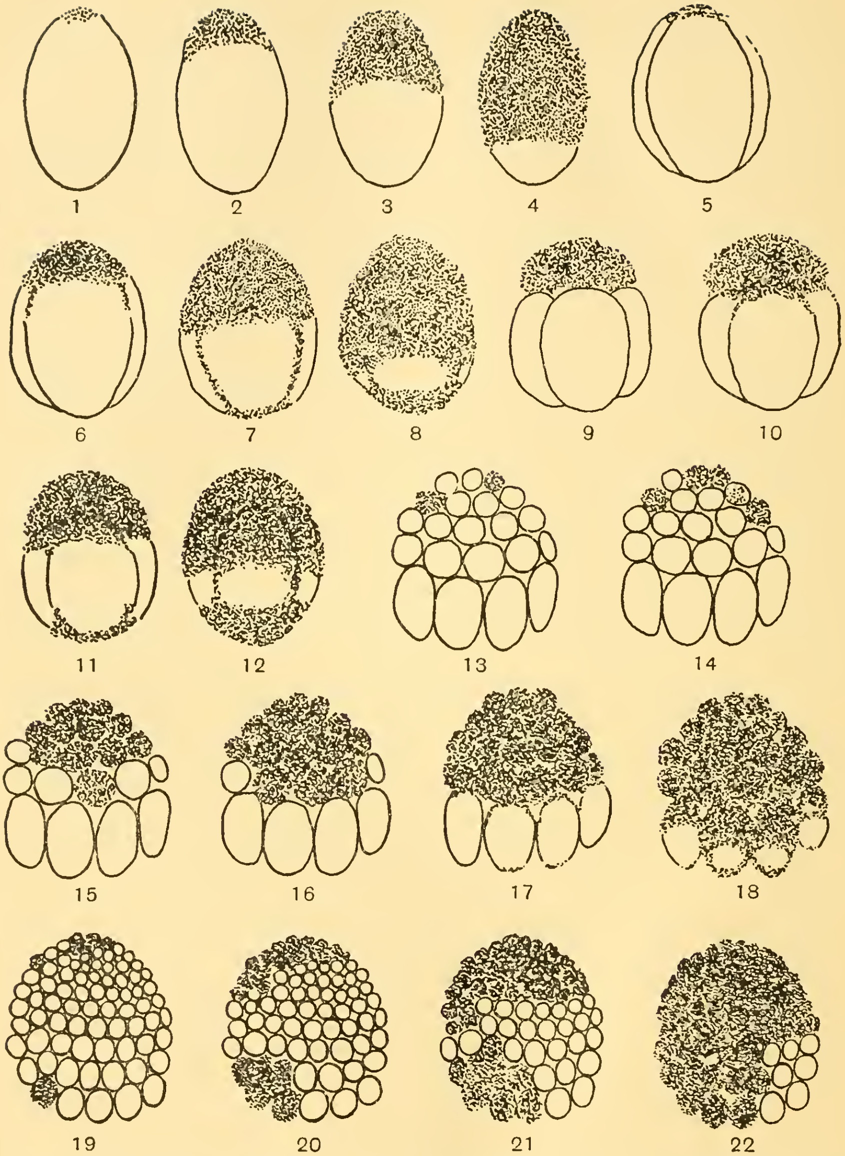
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Fig. 100. Physiological gradients in the egg of the lamprey (Hyman). 1–4. Unfertilised egg. 5–8. Four-cell stage. 9–12. Eight-cell stage. 13–18. About thirty-two-cell stage, 16 hours. 19–22. Morula, 20 hours.

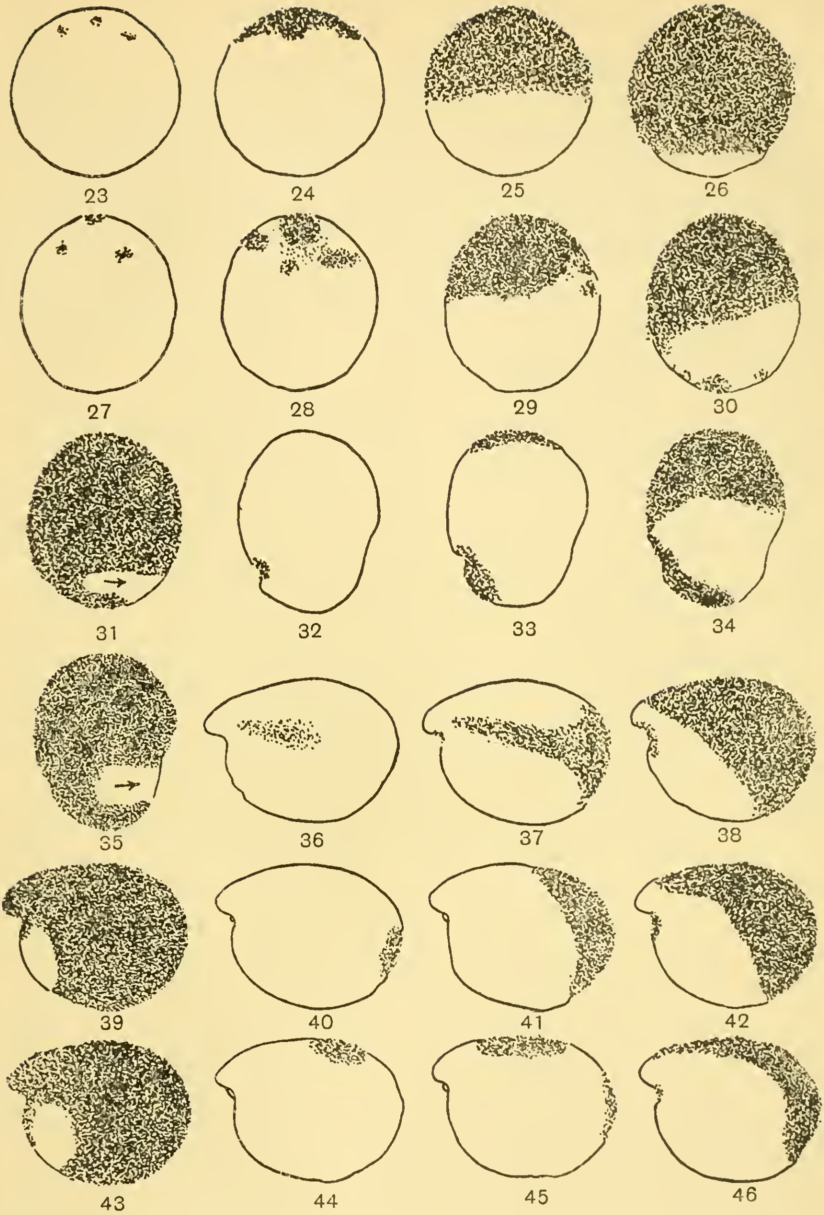
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Fig. 101. Physiological gradients in the egg of the lamprey (Hyman). 23–26. Early blastula, 24 hours. 27–31. Late blastula, 40 hours. 32–35. Beginning of gastrulation, 48 hours. 36–39. Gastrula, 60 hours. 40–43. Late gastrula, 70 hours. 44–46. Late gastrula, neural groove about to appear, 75 hours. From no. 36 onwards, the anterior end is to the right.

for producing differential death. An interesting point was that the embryos were first stained with neutral red, and the change in tint of this indicator noted as soon as the killing solutions were poured on them, obviously showing that the differences in killing time of different parts were not entirely due to differences of permeability. Cannon and Huxley were at one time inclined to attribute most of the results obtained by the direct susceptibility method to such differences. But in any case differences of cell-membrane permeability would be included in the variables which might be changing along the physiological gradient.

The disintegration gradient of the unfertilised lamprey egg was found to be a perfect example of a simple primary gradient, the degeneration beginning at the animal pole and spreading regularly to the vegetative pole. At the 4-cell stage the disintegration begins at the animal tips of the four blastomeres, and passes backwards to meet a slight secondary zone of high susceptibility at the vegetative ends. These changes are seen in Figs. 100 and 101, taken from Hyman's paper. In the 8-, 12- and 16-cell stages, the degeneration begins in the micromeres at the animal pole, then passes on through the macromeres. In later stages the disintegration constantly begins at the animal pole, but in addition isolated cells or groups of cells are to be seen in either animal or vegetal hemisphere which disintegrate in advance of the region in which they are situated. Hyman supposed that these were taken by the killing solution in the act of cleavage, and were thus more susceptible than their neighbours. The secondary region at the vegetal pole is now disappearing for good. The early blastula stages show the usual single gradient, but an important change occurs in the later blastulae, namely, that the spread takes place more rapidly along one surface of the egg than the others. This foreshadows the differentiation of that surface as the dorsal surface. In very late blastulae, there is a small zone of susceptibility near the vegetal pole, which foreshadows the gastrular invagination. The gastrula stages, as shown in the figures, are characterised by disintegration beginning at the anterior end of the embryo and proceeding backwards, but first dorsally and then ventrally to meet the spread from a secondary zone originating around the blastopore. These conditions continue unchanged during the formation of the neural groove and the neural tube, though in the late stages of the latter there is a slight double gradient in it. No further changes occur during elongation and hatching.

The gradients in the chick embryo were also studied by Hyman, using potassium cyanide, and ammonium and sodium hydroxides, sometimes preceded by staining with neutral red. The earliest stages were very difficult to deal with, but some evidence was obtained of an antero-posterior gradient in the central opaque area of the germinal disc at 7 hours' incubation. This was quite certainly demonstrable, however, at the typical primitive streak stage, and the stage of the head process. The medullary plate stage marked the beginning of the double gradient (see Fig. 102), two regions of high susceptibility being present, one at the anterior end of the primitive streak and the other at the anterior end of the medullary plate. When the first somites appear the same zones are seen, the former spreading backwards and forwards along the embryonic axis, the latter backwards only. As the neural folds close, they present a region of high susceptibility, but this soon disappears, and the embryo reverts to the simple double antero-posterior gradient system. This holds good up to the 8-somite stage; from the 9th onwards the rapidly increasing susceptibility of the optic cups is noticeable. At the 12-somite stage the optic zone has died away and there is a new one of high susceptibility in the hind brain, foreshadowing the turning of the head, but this also disappears by the 3rd day of development. Summing up the results, one may say that the general picture is one of an antero-posterior gradient complicated from time to time by the appearance of zones of high susceptibility at different points along the embryonic axis.

In vertebrate embryos in general, it would seem that the formation of these two regions of high susceptibility is the regular mode of development. The two centres are always located in the same position with respect to the future embryo, one at the anterior end of the antero-posterior axis and one in the axis at a more or less posterior point. This posterior centre is the dorsal lip of the blastopore in cyclostome and amphibian embryos, the posterior end of the embryonic axis in teleostean fishes, and the primitive knot, subsequently the tail bud, in the chick. "This posterior centre", says Hyman, "is like a growing point which, passing backwards, deposits the trunk of the embryo anterior to it." The presence of two centres of activity was long ago recognised in frog and rabbit embryos

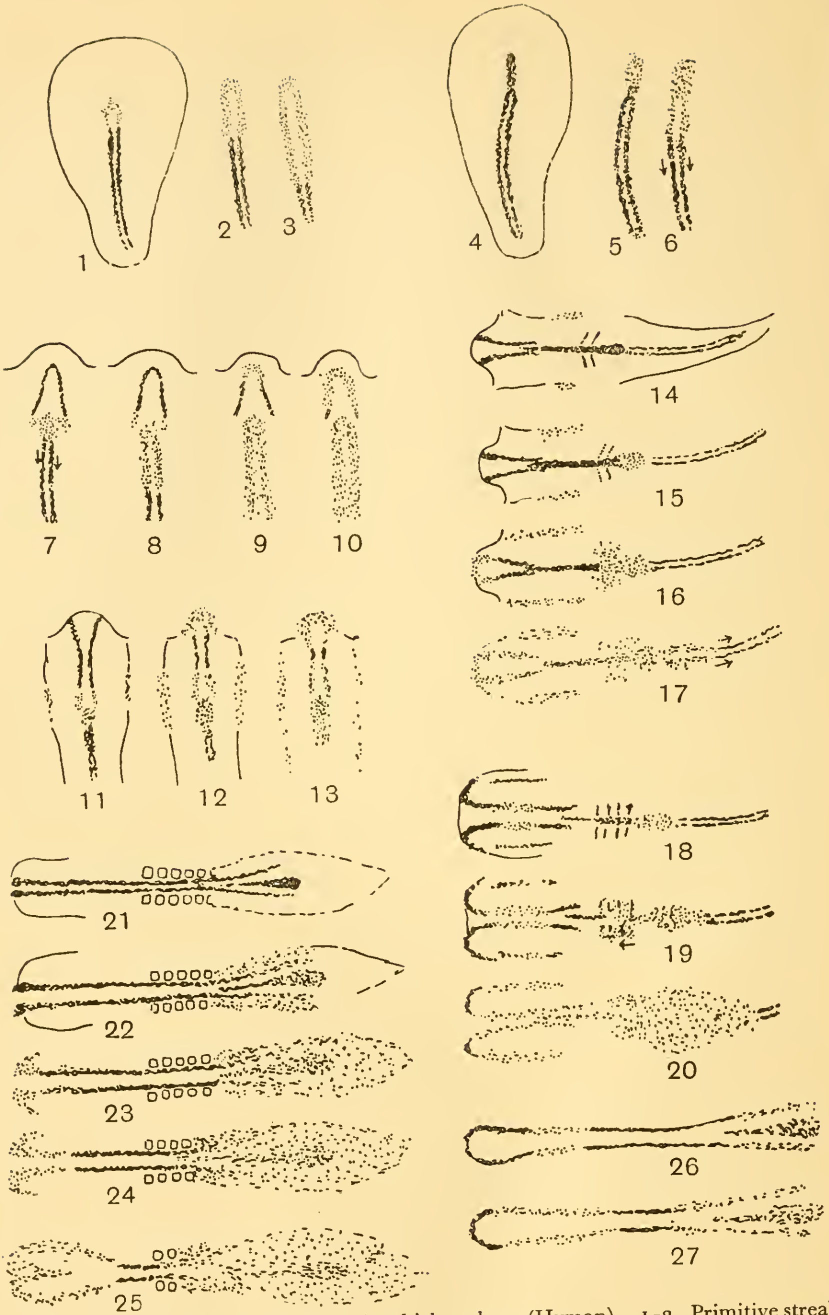
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Fig. 102. Physiological gradients in the chick embryo (Hyman). 1–3. Primitive streak stage. 4–6. Head-process stage. 7–10. Medullary plate stage. 11–13. Early neural fold stage. 14–17. One-somite stage. 18–20. Three-somite stage. 21–25. Five-somite stage. 26–27. Five-somite stage showing heightened susceptibility of point of fusion of neural folds.

by Assheton, who referred to them as primary and secondary centres of cell-proliferation, and this point of view has been adopted by many embryologists, e.g. Eccleshymer; Adelmann; and Kingsbury.

In addition to these researches on embryos, Child has studied the gradients during the development of the sea-urchin egg (*Arbacia punctulata*), those of polychates (*Nereis*, *Chaetopterus* and *Arenicola*) and those of an ascidian (*Corella willmeriana*). In these cases, the primary simple apicobasal gradient of the fertilised egg-cell was succeeded by a double gradient resulting from the appearance of a zone of high susceptibility at the posterior end of the embryo. Mention of the work of Bellamy on the amphibian egg brings us back to our starting-point, namely, the recent investigations on the organiser and the mechanics of amphibian development. There is no need to give a detailed description of Bellamy's results on the frog embryo, for they resembled in many ways those of Hyman on the brook lamprey. But they may be briefly summarised for the purpose of comparing them with the work of Spemann and his school.

Bellamy found that in the unfertilised amphibian egg the beginnings of polarity were to be found in the position in the ovary. He observed by injections and by actual observation of blood-flow that the blood-vessels to the eggs in the oogonia pass arterially over the pigmented part of the egg and venously over the unpigmented part. It is more than probable that the first polarity of the egg arises because the animal pole is that point on the surface of the egg which happens to be most well supplied with a capillary network, not that which happens to be attached to the ovary by the pedicle. The initial physiological gradient, therefore, would seem to be a matter of position in the ovary. It may be mentioned here that very similar conclusions were come to by Lillie for *Chaetopterus* and *Sternapsis* eggs, by Child for *Phialidium* (hydromedusa) eggs, and by Boveri and Jenkinson for *Strongylocentrotus* eggs.

The early stages of development in the frog's egg are very resistant to toxic agents. But it was possible to show that the fertilised but undivided egg began to disintegrate at the animal pole and the degeneration passed downwards and outwards with a special bias towards the grey crescent. Much the same state of affairs was seen in the 4-cell stage, but in the morula stages there are two zones of high susceptibility, the second one appearing just above the grey crescent, and contributing to the general spread downwards from the animal pole. Eggs in an early gastrula stage always disintegrated first at the dorsal lip region and shortly afterwards in the same meridian about 120 to 130° above the blastopore. From the upper point at the animal pole the wave spreads downwards, and meets the disintegrated area of the dorsal lip, which has spread apically and now includes the lateral lips, after which all the pigmented cells become gradually involved, though the cells at the vegetal pole retain their structure with their yolk long after the rest of the egg has died. Later on, when elongation has begun, the dorsal lip now takes up the posterior position, and the double gradient still persists, disintegration beginning from both ends, from the apical point at the anterior end and from the dorsal lip at the posterior end. This description applies to most of the later stages, including the time of opening and closing of the neural folds, the appearance of ventral suckers, etc. Eventually, with the differentiation of various organs, local susceptibility differences begin to appear, and the tail bud, the optic vesicles, the nasal pits, and other rapidly growing regions show much susceptibility. (See Fig. 103.)

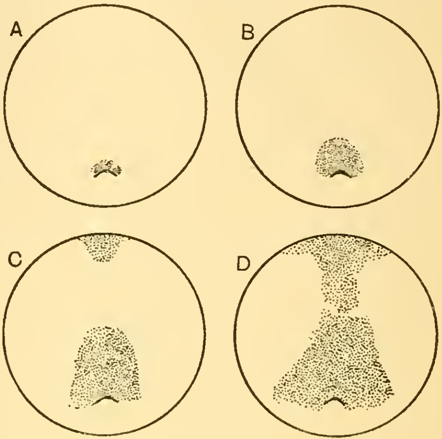

Fig. 103.

On the basis of these fundamental results, Bellamy was able to make and verify teratological predictions, and to control experimentally or modify development, e.g. the gastrular angle and the cleavage ratio (the ratio between the sizes of animal and vegetal pole cells). He was criticised by Cannon, whose main objection was that the effects of the toxic agents were not uniform at the different stages, but that the individual differences between eggs were so large as to invalidate Bellamy's conclusions. When Cannon did succeed in getting a lot of eggs to behave in the same way at the same time, he found results quite at variance with Bellamy's, e.g. the ventral, not the dorsal, region of neural tube stages was the more highly susceptible. These criticisms were replied to in detail by Bellamy & Child, who successfully rebutted them, and whose paper should be consulted for further details. Other criticisms of the general theory of physiological gradients have been made by Wilson; Kingsbury; Lund; Allen; and others, but they do not affect the main conclusions which have been described.

The significance of Huxley's remarks in 1924, which have already been quoted, can now be better appreciated. At the time of chemodifferentiation, the various irreversibly determined regions differ from each other by the presence not only of qualitatively different substances but also by the presence of varying concentrations of the same substance, according to the conception of axial gradients. "If it is asked", said Huxley, "how we can imagine the process as originating, the answer must, I think, follow some such lines as these. During gastrulation every portion of the embryo has a definite relation to the system of axial gradients. The two main gradients extend both on the surface and internally and together constitute a three-dimensional system of gradient co-ordinates. Every portion of the embryo, therefore, has its own rate of activity corresponding to its position in the existing co-ordinate system, and its own characteristic proportions of yolk, glycogen, cytoplasm, etc., depending on the previous effects of the apicobasal gradient during the growth of the egg. When the organiser in the dorsal lip exerts its admittedly as yet unexplained though not unparalleled action of initiating differentiation, every region of the embryo is in a different condition from every other. The substrate is different from place to place, the result, therefore, also differs." The position of a given point in the embryo on the physiological three-dimensional graph is thus of greater importance than the proportions of primary materials which it contains, and, further, the relative velocities of processes going on there are more important than the actual amount of substances of different kinds that happen to be present there. The substances which can be distinguished in the unsegmented ovum of a vertebrate are thus merely raw materials, and the organising influences are to a large extent expressible in terms of gradients of activity. The first of these is probably determined before the egg is laid at all, the second arises from the action of agencies external to the egg approximately at fertilisation. We are thus left with the conception of parts of the embryo as pacemakers of growth and differentiation relatively to the rest: though in what exactly their influence consists we do not as yet know. "The first step in organisation and in embryonic development", says Child, "results from the establishment in one way or another of some region or portion of this protoplasmic reaction-system as a region of higher rate of dynamic activity. This region dominates development, becomes the apical or head region and determines the axial gradient or gradients which constitute the dynamic basis of polarity and of individuation."

3·9. Organised and Unorganised Growth

An idea which has much importance for the study of the interrelations between growth and differentiation was contained in a paper by Faris on the pigmentation of *Amblystoma* embryos. The details of his investigations into the ontogenesis of this pigment will be referred to again in the section on pigments; here I am concerned to refer to his distinction between "proliferation metabolism" and "differentiation metabolism". He observed that, during the development of the myotomes of *Amblystoma* embryos, the pigment accumulated in the cells proportionally to differentiation and not to growth. He therefore suggested that it could be regarded as an index of the difference in nature between the type of metabolism associated with growth and that associated with differentiation, admitting, of course, that the two processes were only completely separable in the abstract. "Proliferation", according to Faris, "must lack the wear-and-tear processes that are characteristic of differentiation and for that reason it lacks the function of pigment production." It must be admitted that these concepts are vague enough, and they rest on an unsatisfactory, because unquantitative, basis. But they have a real interest, in view of two lines of recent thought: firstly, the distinction made by Murray between the groups of processes in embryonic development according to velocity at different times, and secondly, the numerous papers of Warburg and his school (see Section 4·20), which introduce into these problems the concepts of organised and unorganised growth metabolism. These investigations will be referred to in detail in the next section. If it should turn out that Faris's conception of two types of metabolism found a quantitative basis in the data of Warburg and his collaborators, an interesting and quite important avenue would be open for further investigation.

The notion of a distinction between organised and unorganised growth had not, however, its sole origin in Germany, for Byerly had also come to it from very different ground. Setting out from the idea originally suggested by Jordan that haemopoiesis was associated with lack of oxygen or accumulation of carbon dioxide, he allowed chick embryos to develop for 24 hours or so, and then, breaking off the shell covering the air-space, immersed the whole egg in water-glass solution so that the respiratory exchange was quite stopped, but incubation allowed to proceed till 96 hours. He then examined the suffocation effects so produced. These were (1) normal body-form only at the anterior end, (2) no allantois, (3) extraordinarily large blood-vessels and anomalous sinuses, (4) constantly recurring fatty necrosis of tissues. Since the circulation of blood had stopped in these embryos, the end-products of metabolism were accumulating in their cells. The enormous amounts of blood found obviously suggested an unusual haemopoietic activity. But the heart, being unable to beat in the toxic anoxaemic blood, allows it to accumulate in the vessels, and, as more blood-cells are continually being formed, sinuses develop. Cessation of the circulation having led to a struggle for existence between the various parts of the body, some may find it possible to live on the rest, and unorganised "anarchistic" or unregulated growth may occur. This is what Byerly actually found in the suffocated embryos, as regards the formation of blood, but he did not bring forward any evidence that an unusual quantity of lactic acid was produced in the suffocated embryos, as would have been the case on Warburg's view if one tissue had taken on an anaerobic life and was growing at the expense of the others. Holmes was later unable to repeat these observations of Byerly's on the chick embryo, but the number of her experiments was insufficient to negative definitely Byerly's conclusions.

In his second paper, he continued the study of "dead" embryos, which he observed to show three types of behaviour. One class remained on the surface of the yolk and showed anarchistic growth, one absorbed liquid to form a bladder-like vesicle on the surface of the yolk, and one sank beneath its surface. Behaviour of the tissues of embryos of the first class varied according to the age of the embryo at the time when the heart was made to stop beating. If cardiac failure occurred after 4 or 5 days' incubation, only the liver cells and certain of the blood-cells were still proliferating at the end of a week's anaerobiosis. But if the failure took place at 72 hours' incubation or less, then, in addition to liver and blood growth, the nervous tissue went on proliferating for nearly a week. In such embryos the mesenchyme cells seemed almost unaffected by the suffocation, and continued to divide, but took on the histological characteristics of blood-cells. The important point about the picture in anaerobic embryos was that each cell became a relatively free unit, and all correlation of growth as well as all further differentiation ceased with the circulation. Only unorganised cell-life was possible.

No consideration of organisation in growth could omit the subject of mitogenetic rays, from which it appears that radiant energy of definite wave-length is given off by cells in mitosis as an excitant to adjoining cells not in mitosis. For detailed information on this subject, the book of Gurwitsch & Gurwitsch and the memoir of Borodin should be referred to. Genuine uncertainty still exists, however, about these rays, and a whole literature is growing up, partly consisting of reports of workers who confirm Gurwitsch's results, and partly of the reports of those who do not.

Anikin has already attempted to relate the activity of these "mitogenetische Strahlen" to organiser phenomena, and Sorin & Kisljak-Statkewitch have examined all the parts of the hen's egg, using onion roots as detectors for the rays. Negative results were obtained with albumen from the 2nd day of development, the vegetal pole of the yolk throughout incubation, the white and yolk of infertile eggs, even if incubated, the "Brei" of germinal spots 36 hours old, cerebro-spinal fluid and brain tissue from 5-day embryos, the amniotic liquid and certain other parts. On the other hand, positive results were always obtained with the substance immediately under the germinal spot up to the 6th day of development and with the blood. Karpass & Lanschina also find peptic and tryptic digests of egg-yolk to be powerful sources of mitogenetic rays.

3·10. Chemical Embryology and Genetics

The relations between physiological and chemical embryology and genetics, on the other hand, afford a more solid basis for discussion. It has frequently been found that the behaviour of organisms of known genetic constitution during their embryonic period affords a means of marking out the points in ontogenesis at which genetic factors come into play*. The simplest case of this kind is the question of embryonic mortality, which will be mentioned again in Section 18, and which provides a striking temporal field for the display of genetic characteristics. To take only one example, Dunn & Landauer studied the relative embryonic mortality of a variety of chick called the "creeper". Cutler had first reported that, in "creeper" fowls, the leg and wing bones were shorter and thicker than in normal fowls, and that creepers never bred true, but usually produced (*a*) normal chicks, (*b*) creeper chicks and (*c*) chicks with extreme leg defects. The case was thus analogous to that of the yellow mouse, which is always heterozygous, because the homozygous yellow embryos die early in development. The relative embryonic mortalities were found to be as follows:

---

* Some embryological factors seem to be determined entirely by the maternal organism (Toyama for pigmentation of silkworm eggs, Diver, Boycott & Garstang for dextrality and sinistrality of *Limnaea* eggs).

Percentage of total embryos incubated.

	Dead in shell			Hatched alive
	1–6 days	7–13 days	14–21 days	
Creeper male × creeper female	45·5	15·2	12·1	27·3
Creeper male × normal female	4·2	16·9	12·7	66·2

These results lead naturally to the assumption that the high early mortality of the creeper × creeper matings was due to the death of homozygous creeper embryos early in development. The creeper variation would thus seem to be due to a single dominant gene which is lethal in the homozygous condition. But the important point for this discussion is that, if the point of action of a gene can be found to occur at a definite point in development, a new outlook in genetics becomes possible, for what we know to be taking place in the physiological and chemical activity of the embryo at that period may tell us a good deal about the gene itself.

Much thought has been given to these questions in recent years. Danforth, for instance, asked the question whether genes interact with one another during embryonic development to produce structural and functional characters, or whether they each exert their influence separately, thus

$$\begin{tikzcd}
 & & S_1 & & A \arrow[r] \arrow[dr] & n \arrow[r] & S_1 \\
\left.\begin{matrix}A\\B\end{matrix}\right\} \arrow[r] & N \arrow[ur] \arrow[dr] & & \text{or} & & n' \arrow[r] & S_2 \\
 & & S_2 & & B \arrow[r] \arrow[ur] & n'' &
\end{tikzcd}$$

Experiments with mice led him to regard the latter view as the more probable. The three known effects of the Y-gene occur at 6, 25 and 90 days after fertilisation of the egg, and a complex which modifies one of these effects may have no effect on the other two, e.g. if colour is replaced by albinism, yellow is entirely suppressed but senile adiposity is not influenced.

Plunkett made a systematic analysis of the way in which genetic factors and environment interacted to produce bristles during the embryonic development of *Drosophila*. These bristles can easily be counted, and so afford a quantitative variable. What actually happens finally as regards bristles depends on (1) genetic factors, e.g. missing-bristle genes, extra-bristle genes, etc., (2) the temperature during the developmental period, (3) other environmental conditions such as nutrition, (4) differences of internal environment and (5) random internal variations. The last two of these can be avoided by suitable methods. Rise of temperature, Plunkett found, tends to suppress bristle formation. It must act, then, either by decreasing some bristle-forming reaction or by increasing some bristle-inhibiting reaction. If the temperature characteristic, he argued, of this reaction is less than development as a whole, the former must hold, but if it is greater, then the latter must hold. As the evidence came out, it was in favour of the former theory, for the critical thermal increment was 36,400, a value often found for heat destruction of enzymes, although for development as a whole it was 27,800 from 14 to 17°, 17,100 from 17 to 25° and 9000 from 25 to 30°. By various calculations, Plunkett showed that the reaction in question began very early in the larval stage, and took place in three steps: (*a*) production of *R* (a destructive agent for the enzyme) from some protoplasmic component, (*b*) destruction of *B* (enzyme from the gene) by *R* and (*c*) the formation of the bristles catalysed by *B*. A bristle-reducing gene might therefore throw its weight into the catalysis of the formation of *R*. Plunkett suggested that all genes act by differential acceleration of enzyme actions during embryonic development. Similar work on temperature characteristics of gene action has been done by Nadler; and Gowen, after X-raying *Drosophila* at various stages of development, reported that the abnormalities produced, being strictly confined to certain groups of cells, showed that the gene had been affected prior to any action, and while it was still, as it were, lying latent in the nucleus.

Huxley & Ford and Ford & Huxley have undertaken interesting studies on the eye-pigment of *Gammarus chevreuxi*. Allen & Sexton had found that the red colour of the eyes in this organism darkened with development almost to black, and, by working with an arbitrary scale of colours, Huxley & Ford were enabled to make a quantitative examination of the rates of action of the various genes which influence eye-colour. Fig. 104, taken from their paper, demonstrates diagrammatically the relationships found. The blackening appears to be due to the deposition of melanin; in some cases this occurs very rapidly, in others more slowly. Thus the steepest curve in the diagram represents the dominant black-eye type which is black at hatching. Embryos of this type, however, which have not completed half their incubation, have no colour in their eyes, but soon they become pale pink and later scarlet. Just before the end of embryonic life, the eyes darken, until at about the time of extrusion from the brood-pouch they are quite black. It has taken 10 days from fertilisation at 20° to bring this about. Other genetic types, however, hatch with red eyes, and only much later in life approach to blackness, as the diagram illustrates for various varieties. Thus, a definite relation was found to exist between Mendelian genes and rate of a chemical process.

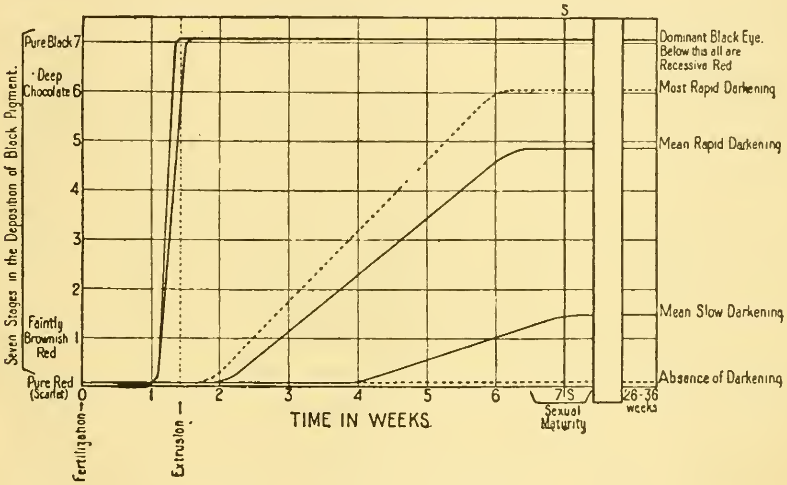

Fig. 104.

Morgan, in 1923, discussed such questions as these. "It is to be hoped", he said, "that in time the combined attack on the problem of development by genetics and experimental embryology and especially by chemistry may lead to the discovery of the physiological action of the genes, but for the present we may confess ignorance." A great step forward in this direction was taken by Goldschmidtt in his important book on physiological genetics. He assumes that genes are primarily of the nature of enzymes or substances which can excite the action of enzymes. Working on the caterpillars of *Lymantria dispar* he found that by crossing the European and Japanese races, intersexes could be obtained, and he was able to identify their various grades between the two poles of maleness and femaleness with the time-process of development, i.e. with critical points earlier or later in ontogeny. His conclusions were, firstly that the velocity of the sex-determining (and all morphogenetic) reactions was proportional to the quantity of the genes present, secondly that all the morphogenetic reactions go on side by side, the most rapid one controlling development and, thirdly, that the morphogenetic reactions involve determination-hormones brought into being by gene action. In other words, the genes evoke the organisers, which evoke the morphogenesis. It is obvious that the co-operation of the chemist and the geneticist in the investigation of embryonic development will be very fruitful in the future. With the work of Onslow on the chemistry of coat-colour and of Brink and his collaborators on the waxy gene in maize and its chemical effects, such co-operation may already be said to have begun. Brink & Abegg, in an important passage, point out that, though at present there is little likelihood of valuable results emerging from further chemical study of nuclear material, i.e. the genes themselves, there is every chance of success in the investigation of the chemical field of action of the genes. The manner in which these units function in ontogeny plainly offers a prodigious field for the future work of chemical embryologists. Interesting reviews of this subject are those of F. R. Lillie and of T. H. Morgan.

This section may fitly be concluded with the words of Sir W. B. Hardy: "Let us consider the egg as a physical system. Its potentialities are prodigious and one's first impulse is to expect that such vast potentialities would find expression in complexity of structure. But what do we find? The substance is clouded with particles, but these can be centrifuged away leaving it optically structureless but still capable of development....On the surface of the egg there is a fine membrane, below it fluid of high viscosity, next fluid of relatively low viscosity, and within this the nucleus, which in the resting stage is simply a bag of fluid enclosed in a delicate membrane. How shall sources and sinks of energy be maintained in a fluid composed of 80 per cent. of water? They are undoubtedly there, for the egg is a going concern, taking in oxygen and maintaining itself by expenditure of energy....The egg's simplicity is not that of a machine or a crystal, but that of a nebula. Gathered into it are units relatively simple but capable by their combinations of forming a vast number of dynamical systems into which they will fall as the distribution of energy varies."

END OF VOLUME I

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